Study for the efficient synthesis of oligosaccharides: alternative sialylation and rapid separation methods

効率的オリゴ糖合成に関する研究：新しいシアリル化法と迅速分離法

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Contents

List of Abbreviations

Ac	Acetyl
Ac_2O	acetic anhydride
AcOH	acetic acid
Ag_2CO_3	silver carbonate
AgOTf	silver trifluoromethanesulfonate
AIBN	2,2′-azobis(2-methylpropionitrile)
Bn	Benzyl
BRSM	based on recovered starting material
Bz	Benzoyl
CAN	ceric ammonium nitrate
CH_3CN	Acetonitrile
ClAzb	4-azido-3-chlorobenzyl
CPG	controlled-pore glass
DCM	Dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	diethyl azodicarboxylate
DIEA	*N*,*N*-diisopropylethylamine
DIPC	*N,N*′-diisopropylcarbodiimide
DMAP	4-dimethylaminopyridine
DMF	Dimethylformamide
DMP	Dess-Martin periodinane
DMTST	dimethyl(methylthio)sulfonium trifluoromethanesulfonate
DVB	Divinylbenzene
EtCN	Propionitrile
Fmoc	Fluorenylmethyloxycarbonyl
FRP	fluorous reverse-phase

GPI	glycosylphosphatidylinositol
$HgBr_2$	mercury (II) bromide
$Hg(CN)_2$	mercury (II) cyanide
^1H NMR	proton nuclear magnetic resonance
HOBt	1-hydroxybenzotriazole
IX	iodine interhalogen
Kdn	2-keto-3-deoxy-nonulosonic acid
MALDI-ToF MS	matrix-assisted laser desorption/ionization time-of-flight mass spectrometer
MeOH	Methanol
MeSBr	methylsulfenyl bromide
MPEG	polyethylene glycol monomethyl ether
MS	molecular sieves
MSNT	1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole
NaOMe	sodium methoxide
NBS	N-bromosuccinimide
Neu5Ac	*N*-acetylneuraminic acid
Neu2en5Ac	5-*N*-acetyl-2-deoxy-2,3-didehydro-neuraminic acid
Neu5Gc	*N*-glycolylneuraminic acid
NIS	*N*-iodosuccinimide
PEG	polyethylene glycol
PhSOTf	phenylsulfenyl triflate
PS	Polystyrene
PTHF	Polytetrahydrofuran
2-PyMeSi	2-pyridyldimethylsilyl
Rt	room temperature

SCX	silica-supported ethylbenzenesulfonic acid
SMe	(methylthio)trimethylsilane
SPOS	solid-phase oligosaccharide synthesis
TBAOH	tetra-*n*-butylammonium hydroxide
TE	2-(trimethylsilyl)ethyl)
TEAH	tetraethylammonium hydroxide
TFA	trifluoroacetic acid
TfOH	trifluoromethanesulfonic acid
THF	Tetrahydrofuran
TLC	thin layer chromatography
TMSCl	trimethylsilyl chloride
TMSOTf	trimethylsilyl trifluoromethanesulfonate
Trt	Trityl
p-TsOH·H_2O	*para*-toluenesulfonic acid monohydrate
UV	Ultraviolet
WSCD·HCl	water soluble carbodiimide hydrochloride (*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride)

Abstract

Oligosaccharides are carbohydrates composed of a small number of monosaccharides usually present as components of glycolipids and glycoproteins. These are not only involved in normal physiological processes but in pathogenesis as well. Sialic acid-containing oligosaccharides in pathogenesis can either function as a marker that the pathogen has already penetrated a vulnerable part of the host's system, or as a decoy of the host's immune system to eliminate the pathogen and prevent virulence. For the synthesis of sialic acid containing oligosaccharide, stereoselective α-sialylation is essential but it has been placed as one of the most difficult glycosylations, since the glycosylation takes place at the sterically hindered position in the absence of participating group that can direct stereoselectivity, and the presence of an electron-withdrawing carboxylic acid at C1 electronically disfavors oxocarbenium ions. The recent advances in glycosylation chemistry, e.g., development of the new leaving groups and protecting groups, enabled the efficient construction of α-sialoside linkages. In the present study, the author developed the new stereoselective sialylation method using a sialyl thioglycoside as a donor and iodine interhalogen compounds together with $In(OTf)_3$ system as activators. The combination of iodine interhalogen compounds with $In(OTf)_3$ was found to be highly reactive to promote the glycosylation under -85°C to afford the desired α-sialyloside with high selectivity (Figure 1).

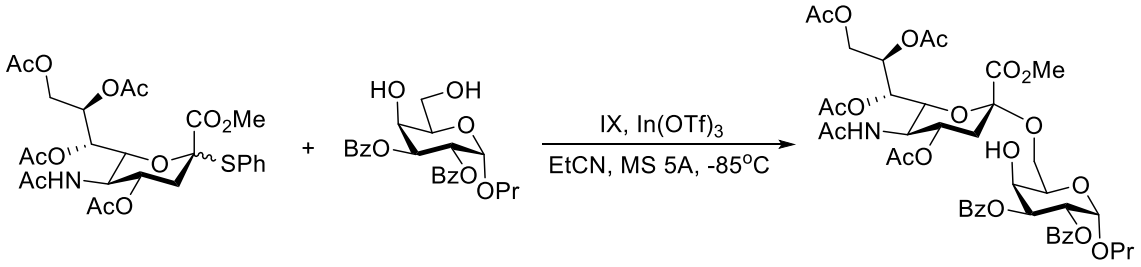


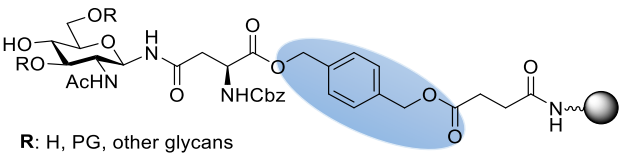
Figure 1. IX/$In(OTf)_3$-promoted α-sialylation.

Because the sialylation sometimes affords by-products such as glycals and hydrolyzed donors, which render the purification of the desired products difficult, the author then investigated the glycan synthesis using phase tag method. One kind of phase tag called affinity tag/label uses specific molecular recognition in facilitating easier separation of the tagged product. By attaching Triton X-100 as a tag to the galactoside acceptor followed by α-sialylation using $ICl/In(OTf)_3$, and finally, passing the reaction mixture through ArgoPore-NH_2 columns, the Triton-tagged disaccharide was obtained. This methodology can be applied to the synthesis of various oligosaccharides as well as other compounds.

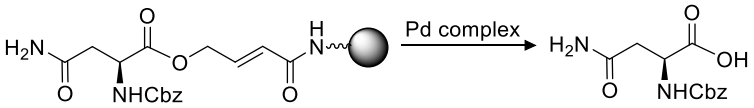
Aside from phase tagging, oligosaccharides can also be synthesized using the solid-phase method. In this rapid separation technique, substrates are attached onto polymer beads prior to the reaction via suitable linkers.

N-glycan synthesis on solid supports have already been reported, however, *N*-glycans linked with asparagine have yet to be synthesized using solid-phase methods. In this study, several benzyl ester-type (Figure 1) and allyl linkers (Scheme 2) have been developed.

Figure 1. Benzyl ester linker.



Linkers were either connected with asparagine prior to resin loading, or directly attached onto the linker followed by asparagine loading. With the benzyl ester linkers, glycosylation was found to proceed on the solid support, however, isomerization of asparagine after basic cleavage was observed. To circumvent this, an allyl linker which is cleavable using palladium complexes was investigated. Allyl linkers were synthesized via cross-metathesis reaction, allylic alcohol oxidation, or direct loading of 4-bromocrotonic acid. Cleavage, on the other hand, were carried out using palladium (II) acetate or tetrakis(triphenylphosphine)palladium(0).



Scheme 2. Allyl linker.

Chapter I Introduction

Oligosaccharides are biomolecules formed by the linkage of a small number of monosaccharides. Owing to the fact that the monosaccharides can be connected in different ways, oligosaccharides are present in diverse structures and are often involved in various biochemical processes. Although studies regarding the functions of oligosaccharides abound, much of the roles that they play within the living organism remain unknown mainly due to difficulties in obtaining pure samples from natural sources. Chemical synthesis of oligosaccharides gave way to circumvent some of these problems, though it is often time-consuming, expensive and resulting in low yields. However, the existence of several hydroxyl groups on not only one, but all of the monosaccharides, requires that these be protected and deprotected at certain stages during the synthesis. Formation of the new glycosidic linkage which has to be stereoselectively controlled, not to mention the required purification at each step remain to be the main hurdles in their synthesis.[1]

In this study, the author investigated the efficient methods for oligosaccharide synthesis. The major targets of the synthesis are sialic acid-containing oligosaccharides, which are known to have very important biological roles. The efficient oligosaccharide synthesis using rapid separation methods were also investigated.

Functions of glcans containing sialic acids

Sialic acid derived from the Greek word *sialos* meaning 'saliva'[1c] as coined by Gunnar Blix, Ernst Klenk and Alfred Gottschalk[1d] encompass a family of monosaccharides having the nine-carbon carboxylated sugar neuraminic acid (5-amino-3,5-dideoxy-D-glycero-D-galactononulosonic acid) as a backbone (Figure I-1). Due to the relative insensitivity and other technical limitations of earlier analysis techniques, the terms sialic acid and *N*-acetylneuraminic acid (Neu5Ac, NANA) have been used as if these were one and the same, and were thought of as species-specific or tissue-specific. At present, Neu5Ac, commonly found in humans, is recognized as the most prevalent form of sialic acid, as well as the precursor of other derivatives. Substitution of the *N*-acetyl group by a hydroxyl group results in the formation of *N*-glycolylneuraminic acid (Neu5Gc), common in many animal species, while the replacement of the whole amino group at C5 by a hydroxyl is called 2-keto-3-deoxy-nonulosonic acid (Kdn), found in fish eggs.[1e] Other modifications include substitutions with acetyl, lactyl, methyl, phosphate or sulfate in the hydroxyl groups, or a double-bond between C2 and C3 such as in 5-*N*-acetyl-2-deoxy-2,3-didehydro-neuraminic acid (Neu2en5Ac) present in the body fluids of a sialuria patient.[1f] It should also be noted that the carboxyl group at C1 makes the molecule negatively charged at physiological conditions, and that C7 acetyl ester groups can also migrate to the C8 or C9 hydroxyl group that is not substituted.[1g] Studies have also showed that in nature, sialic acids are almost only present in vertebrates, or

some protozoa, viruses, and bacterial strains as terminal, non-reducing ends of surface membrane carbohydrates .[1]

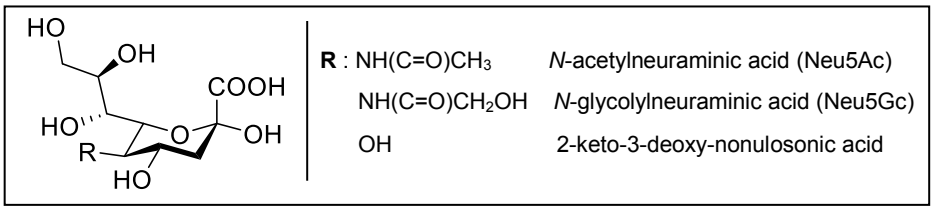


Figure I-1. Representative sialic acid structures.

Over the course of several decades, research about the function of these compounds have been quite extensive, although some areas are still in need of conclusive evidence. In human systems, sialic acids mostly exist as part of gangliosides and glycoproteins, the former being more concentrated in brain grey matter than any other internal organs. Human body fluids such as saliva, serum, urine, and even human milk also contain sialic acids.[1c] Colostrum contains the highest concentration of human milk oligosaccharides,[2a] which is mostly made of sialyllacto-*N*-tetraose, 6'-sialyllactoses, and disialyllacto-*N*-tetraose.[2b] Comparison of the sialic acids in human milk as opposed to bovine milk, which is commonly used in infant formula, revealed that the former consists of Neu5Ac connected to galactose by an alpha 2-6 linkage while the latter contains both Neu5Ac and Neu5Gc in mostly alpha 2-3 linkages with galactose.[1c] Sialic acid was also found to be lower in infant formula than human milk with the sialic acids mostly bound to proteins in the former as compared to free oligosaccharides in the latter.[2c] In a study which analyzed the brain frontal cortex of breast-fed and formula-fed infants who died of sudden death infant syndrome, it was found that the former had higher ganglioside-bound and protein-bound sialic acids which implies that nutrition may affect the incorporation of sialic acids into brain grey matter. This led to the conclusion that sialic acid and long-chain polyunsaturated fatty acids might be codependent building blocks for neural tissues.[2d] It is also interesting to note that the carbohydrates which make up human milk oligosaccharides namely, glucose, galactose, *N*-acetylglucosamine, *N*-acetylgalactosamine, fucose, and sialic acids, are the same ones that make up gangliosides which are very abundant in the brain.[2e] Gangliosides, which consist of a hydrophilic sialyl oligosaccharide and a hydrophobic ceramide tail, have been considered to contribute in the formation of memory due to its calcium ion binding property. In an investigation using newborn piglets supplemented with a diet of varying doses of sialic acid, it was found that the amount of sialylated glycoproteins in the frontal cortex as well as cognitive function were dependent on the dose of supplemented sialic acid. Other studies where gangliosides were used to treat certain central nervous system lesions as well as associating it with the growth and development of neurons further underscores the importance of sialic acids in brain development and cognition.[2f,g]

Given the fact that sialic acids are present on human cell surface as well as in some bacteria and viruses, its link to pathogenesis is unavoidable. Sialic acids on host environment may either act as recognition sites signaling that the bacteria has already penetrated an area that is viable for colonization or as decoys where pathogens can bind and be easily eliminated from the body, thus preventing virulence.[1d,3] Sialic acids as masks of target receptors play crucial roles especially during the early stages of human life where the immune system is not yet fully developed. As previously mentioned, sialic acids abound in human milk oligosaccharides, the primary source of nutrition after birth. Pathogens then bind to these sialic acids and are subsequently, eliminated from the body.[1c] This function is also shared by erythrocytes in the blood stream, mucins in the mucosal surface and other highly sialylated glycoproteins in the plasma and extracellular fluids. After gaining access to these areas, viruses are met with sialylated glycoproteins where they bind and then removed from the host's system ridding it of potential harm. On the other hand, it has been found that throughout evolution, some bacteria have adapted a primitive way of synthesizing nonulosonic acids and modified it to produce vertebrate-like sialic acids which can trick the host's immune system and avoid detection by antibodies. Pathogens can also obtain sialic acids from the host by secreting sialidases or by utilizing free sialic acids cleaved by host sialidases themselves during inflammation. Some of these sialic acids may either be used as carbon and nitrogen sources, or incorporated into pathogen cell-surface macromolecules. When bacterial cell-surfaces incorporate sialic acids, these are able to mimic host cell-surfaces that are rich in sialoglycoconjugates, thus avoiding detection of the host's immune system, as in the case of *Neisseria meningitidis's* resistance against human serum or interaction with sialoadhesins.[3]

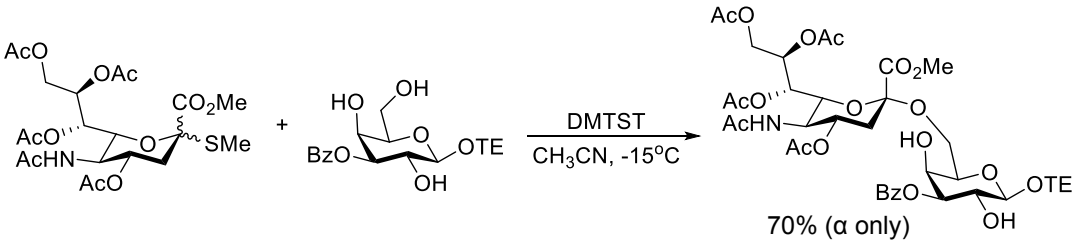
Aside from infections, disruptions in sialic acid metabolism is also implicated with several conditions such as sialuria (excessive production of sialic acid that is excreted through the urine), brain impairment, schizophrenia (the severity of psychosis was linked to lower sialic acids bound to glycoproteins in the cerebrospinal fluid), Alzheimer's disease, and even cancer (where significant amounts of Neu5Gc are detected and increased sialylation maybe modulatory in the adhesion and survival of metastatic cells).[1c,3c] Such are the importance of sialic acids that these have been the target of organic synthesis since the early 1900s.

Sialylation

Nowadays, sialic acids are not only synthesized in order to study their functions but also gauge their potential as therapeutic agents. However, there are complexities inherent in the structure of sialic acids that should be carefully considered prior to their synthesis and glycosylation. The presence of several 2° hydroxyl groups and 3° anomeric centers that greatly affect the kind of protecting groups to be employed, the carboxylic acid that destabilizes oxocarbenium ion formation, the sterically hindered C2 position as well as the absence of neighboring group participation in C3 all lead to poor stereoselectivity and increased side-product formation.[4a]

One of the classic examples of glycosylation involving sialic acids utilizesd β-glycosyl halides using the Koenigs-Knoor reaction in either S_N1 or S_N2 fashion where polar and nonpolar solvents favor each mechanism, respectively. This reaction uses Ag (I) to activate the anomeric center and mainly yields the α-isomer as the product, probably because the leaving group blocks access to the top face of the molecule by the incoming nucleophile. In Helferich methodology, Hg(II) is used instead of Ag(I) in non-polar solvents due to the former's better solubility.[4] Furthermore, it was found that Ag(I) as a promoter is only compatible with hydroxyls having high reactivities.[5] In most of these transformations, 2-chloro Neu5Ac methyl ester derivative were used. Promoters for *O*-glycosylations other than AgOTf, Ag_2CO_3, $HgBr_2$, and $Hg(CN)_2$ have also been discovered. These include polymer-based Ag salts such as silver polymaleate and silver salicylate, and mild Lewis acids such as ZnX_2 (X= Br, Cl, I, OTf) and SnX_2 (X= Cl, OTf). The use of phase-transfer catalysts also provided a cheaper, relatively safer, and faster route to aryl glycosides with higher yields and selectivities.[5]

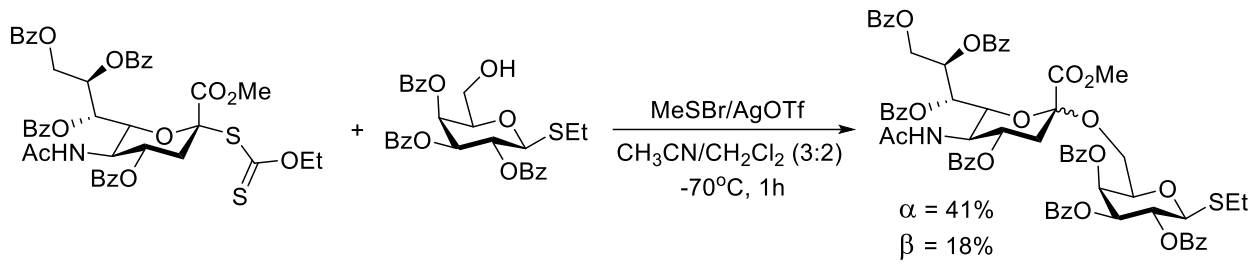
However, due to difficulties in the purification and handling of β-glycosyl halides, sialyl thioglycosides were developed as more stable and versatile sialyl donors. Common promoters used for activation of sialyl thioglycosides were non-metal compounds such as dimethyl(methylthio)sulfonium trifluoromethanesulfonate (DMTST), and *N*-iodosuccinimide (NIS).[4] Most prominent discovery for stereoselective sialylation is the α-directing solvent effect of nitrile found by Prof. Hasegawa and his colleagues (Scheme I-1).[6] Many sialylated glycans have been synthesized by using various silalyl donors in addition to sialyl thioglycosides. However, the yields and selectivity varied depending on the substrates and conditions to sometimes give the desired sialosides with relatively poor yields and selectivity.



Scheme I-1. Glycosylation using DMTST. (SMe: (methylthio)trimethylsilane, TE: 2-(trimethylsilyl)ethyl)

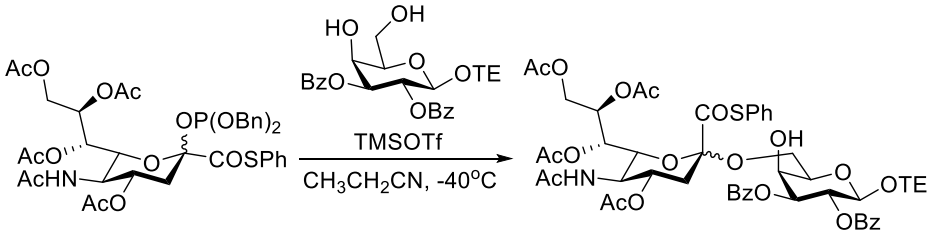
Aside from thioglycosides, 2-xanthates or 2-(ethoxy)dithiocarbonate derivatives of Neu5Ac have also been used as donors in *O*-glycosylations involving sialic acid as a building block. The use of methylsulfenyl bromide (MeSBr)/AgOTf as a promoter can selectively activate 2-xanthates even in the presence of thioglycosides allowing the direct condensation of *S*-glycosyl xanthate as donor with a thioethyl glycoside acceptor as reported by Birberg and Lönn (Scheme I-2).[7b] Other promoters that can be used to activate 2-xanthates include DMTST and phenylsulfenyl triflate (PhSOTf).[7] β-Sialophosphites as donors were also reported to result in high yields

and stereoselectivities requiring only catalytic amounts of promoters such as Trimethylsilyl trifluoromethanesulfonate (TMSOTF). [4a]



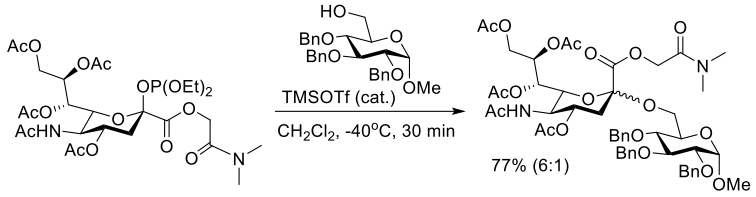
Scheme I-2. 2-Xanthates as donor in sialylation.

Over the years, findings regarding promoters, leaving groups, and auxiliaries in simple *O*-glycosylations also found their way in α(2-6) and α(2-3) sialylations with galactose derivatives. Phenylthioester derivatives of sialic acid can be activated by TMSOTf in α(2-6) sialylations with a galactose or glucose acceptor in high selectivities and relatively high to moderate yields, respectively (Scheme I-3).[8]



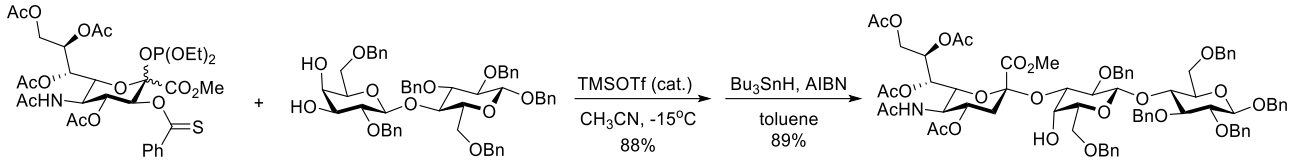
Scheme I-3. Phenylthioester derivative as donor in sialylation.

Efforts in modifying certain areas within the sialyl donors have also been undertaken. One such example is the use of *N,N*-dimethylglycolamide ester auxiliary in C1, which increased α-selectivity by stabilizing the oxocarbenium intermediate while blocking the β-face of the molecule (Scheme I-4).[4b] Sialyl chlorides, sulfides, and phosphites were used in the investigation and it was found that this auxiliary allowed reactions to proceed with better efficiencies than when the unmodified methyl ester donors were used.



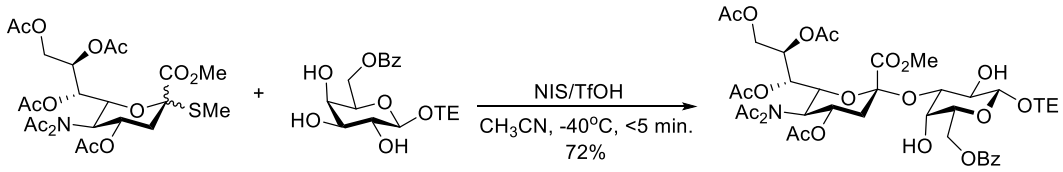
Scheme I-4. *N,N*-dimethylglycolamide ester as C1 auxiliary.

Aside from the C1 position, the C3 position was also a target of the installation of auxiliary groups. One notable design was the attachment of an aryloxythiocarbonyl at C3, the presence of which can block one side of the molecule leaving on the α-face for nucleophilic attack while the anomeric phosphite group can facilitate initiation with catalytic amounts of promoter (Scheme I-5).[4c]

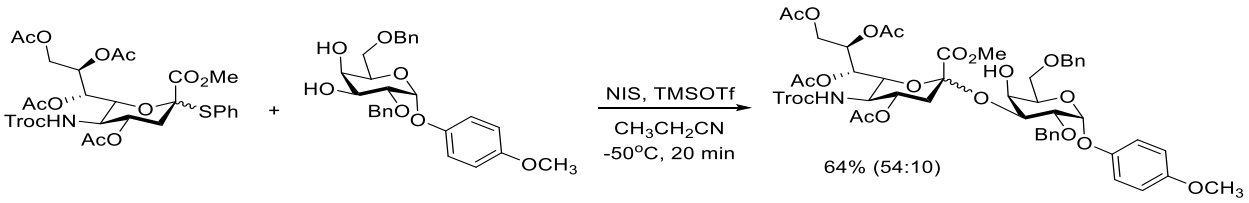


Scheme I-5. Thiobenzoyloxy group as C3 auxiliary.

The various C5-substituents in the sialic acid donors, such as carbamates, have been also developed to tune the reactivity of the oxocarbenium ions. They include *N,N*-diacetamide (Scheme I-6),[4d] *N*-TFA,[4e] azide,[4f] and N-Troc (Scheme I-7)[4g-i] groups and show the improved yields and α-selectivity.

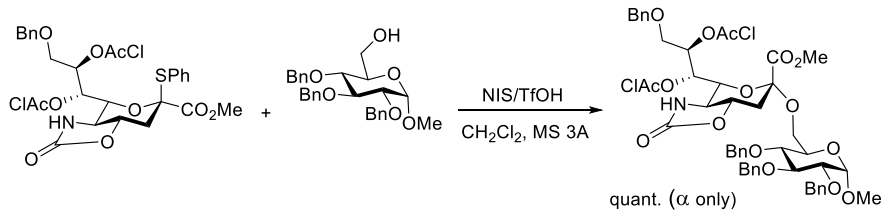


Scheme I-6. *N*-diacetamide modification at C5.



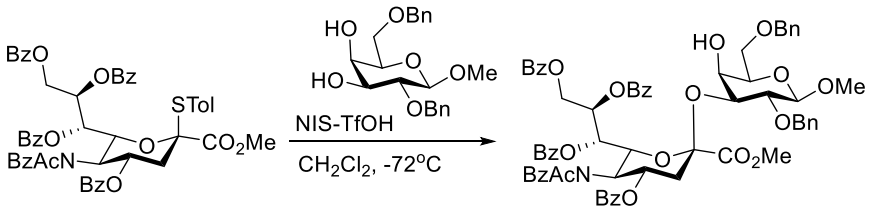
Scheme I-7. *N*-Troc group modification at C5.

The author's laboratory have also developed the *N*-phenyltrifluoroacetimidate donors with C5-phthalimide[4j,k] or azide[4l] groups in applying the concepts of "fixed dipole moments effects", and achieved in the quantitative sialylation with complete α-selectivity under the microfluidic conditions. The oxazolidinone protection involving C4 and C5 of sialic acid as independently explored by Takahashi (Scheme I-8)[4m,n] and Crich[4o] was also notable because of the efficient synthesis of α-di- to tetrasialic acids even without the use of nitrile solvents.[4m,n,p]



Scheme I-8. Oxazolidinone protection at C4 and C5 of sialic acid.

On the other hand, unnatural-type β(2-3)-sialosides were prepared with *N,N*-acetyl, benzoyl-*O*-perbenzoyl protected sialyl donors by using either Ph_2SO-Tf_2O or NIS-TfOH as promoters. Solvent effect was observed for DCM while mixed solvents of CH_3CN/DCM and Et_2O/DCM failed to activate the donor (Scheme I-9).[9]



Scheme I-9. β(2-3) Sialylation involving *N,N*-acetyl, benzoyl-*O*-perbenzoyl protected sialyl donors.

While various sialyl donors have been developed, much attention has not been paid to the *N*-Ac derivatives, the simplest sialic acid donors in a view of the accessibility from the commercial source. While in a few cases, such as α(2-6)-sialylation with the galactose acceptor, *N*-Ac imidate is reported to show high α-selectivity,[10] the most of the literature precedent including our earlier report[4j] showed the poor reactivity of N-Ac donors, which resulted in the modest yields and α-selectivity (~50% and α:β ~ 3 : 1). During our recent investigation of α-sialylation under microfluidic conditions (Figure I-2),[4k,l] we realized that in the conventional flask reaction, the heat generated during the syringe-addition of the Lewis acid to the donor and acceptor, is quite sensitive to the efficiency and reproducibility. We hypothesized that, for the *N*-Ac case, this point has not been investigated to greater depth; namely, *N*-Ac donor would potentially show the good sialylation reactivity, but the local temperature raise owing to heat generation during the inefficient mixing in flask may have consequently led to the poor reactivity and reproducibility. Therefore, C5-acetamide donor was reinvestigated for practical α-sialylation. By efficiently mixing the *N*-phenyltrifluoroacetimidate donor with an appropriate amount of TMSOTf under strict temperature control at -80°C, the α(2-6) and α(2-3)-sialylation were achieved with galactose and glucosamine acceptors in excellent yields and with high α-selectivity.[11]

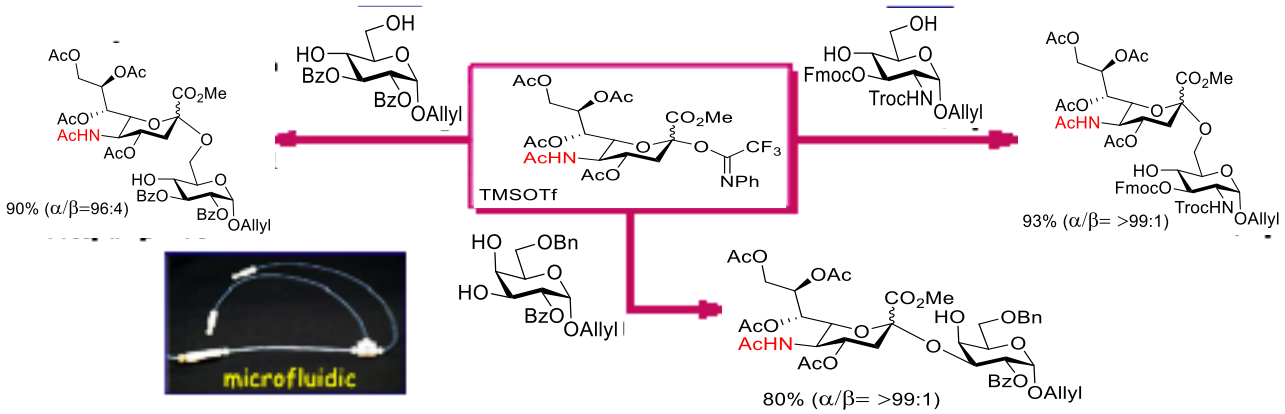
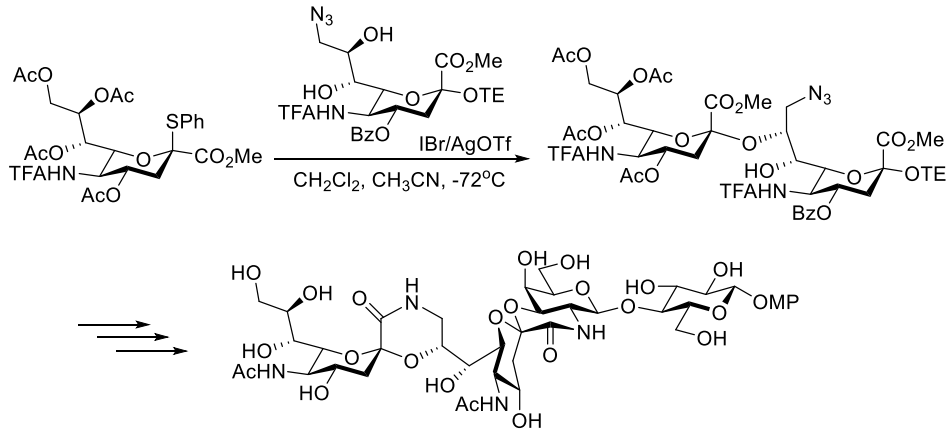


Figure I-2. α-Sialylation under microfluidic conditions.

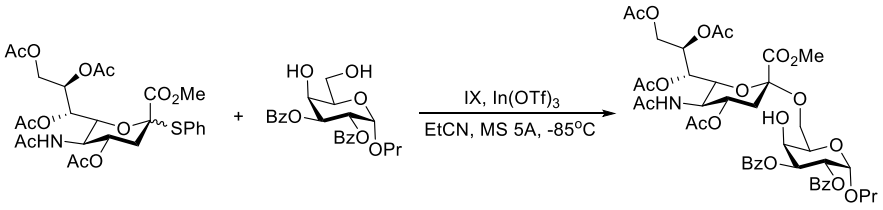
As mentioned above, sialyl thioglycosides have often been used for the synthesis of various sialylated glycans. However, because of the poor reactivity of *N*-Ac thioglycoside donors, activation of the thioglycosides with NIS-TfOH has been usually carried out at -40~-10°C to give the desired sialosides with the modest yields and α-selectivity in general (~50% and α:β ~ 3 : 1). In the present study, the aurthor paid attention to interhalogens such as iodine monochloride (ICl) and iodine monobromide (IBr) as the more reactive halogenated reagents than NIS.

Interhalogens such as iodine monochloride (ICl) and iodine monobromide (IBr) together with AgOTf have also been reported to activate thioglycoside donors in sialylations. The ICl/AgOTf system was found to promote glycosylations where donors have participating groups while the IBr/AgOTf system was better in cases where a participating group was not present, and thus, was suitable for the synthesis of bislactam ganglioside GD3 analogues (Scheme I-10).[12]



Scheme I-10. Synthesis of bislactam GD3 analogues using IBr/AgOTf promoter system.

In the present study, the author found that combination of ICl or IBr with $In(OTf)_3$ showed higher reactivity than IBr/AgOTf for activation of sialyl thioglycosides. The glycosylation reaction was carried out at -80°C by using ICl/$In(OTf)_3$ ICl or IBr/$In(OTf)_3$ as activators (Scheme I-11). The desired sialyl-α(2-6)galactose was thus successfully obtained in a good yield with high α-selectivity. The detail was described in Chapter II.



Scheme I-11. IX/$In(OTf)_3$-mediated sialylation.

References:

1. (a) Weishaupt, M.; Eller, S.; Seeberger, P. *Methods Enzymol.* **2010**, *478*, 463-484; (b) Plante, O. J.; Palmacci, E. R.; Seeberger, P. H. *Science*, **2001**, *291*, 1523-1527; (c) Wang, B.; Brand-Miller, J. *Eur. J. Clin. Nutr.* **2003**, *57*, 1351-1369; (d) Blix, F. G.; Gottschalk, A.; Klenk, E. *Nature* **1957**, *179,* 1088; (e) Nadano, D.; Iwasaki, M.; End, S.; Kitajima, K.; Inoue, S.; Inoue, Y. *J. Biol. Chem.* **1986**, *261*, 11550-11557. (f) Kamerling, J. P.; Vliegenthart, J. F. G.; Schauer, R.; Strecker, G.; Montreuil, J. *Eur. J.Biochem.* **1975**, *56*, 253-258; (g) Kamerling, J. P.; Schauer, R.; Shukla, A. K.; Stoll, S.; Van Halbeek, H.; Vliegenthart, J. F. G. *Eur. J. Biochem.* **1987**, *162*, 601-607; (h) Schauer, R. *Glycoconjugate J.* **2000**, *17*, 485-499; (i) Varki, A. *Glycobiology* **1992**, *2*, 25-40; (j) Traving, C.; Schauer, R. *Cell. Mo. Life Sci.* **1998**, *54*, 1330-1349.
2. (a) Coppa, G. V.; Gabrielli, O. Pierani, P. Catassi, C.; Carlucci, A.; Giorgi, P. L. *Pediatrics* **1993**, *91*, 637-641; (b) Coppa, G. V.; Pierani, P.; Zampini, L.; Carloni, I.; Carlucci, A.; Gabrielli, O. *Acta Pediatr.* **1999**, *Suppl. 430*, 89-94; (c) Wang, B.; Brand-Miller, J.; McVeagh, P.; Petocz, P. *Am. J. Clin. Nutr.* **2001**, *74*, 510-515; (d) Wang, B.; McVeagh, P.; Petocz, P.; Brand-Miller, J. *Am. J. Clin. Nutr.* **2003**, *78*, 1024-1029; (e) Wiegandt, H. *Prog. Brain Res.* **1994**, *101*, 63-73; (f) Wang, B. *Adv. Nutr.* **2012**, *3*, 4655-4725; (g) Wang, B.; Yu, B.; Karim, M.; Hu, H.; Sun, Y.; McGreevy, P.; Petocz, P.; Held, S.; Brand-Miller, J. *Am. J. Clin. Nutr.* **2007**, *85*, 561-569.
3. (a) Varki, A.; Gagneux, P. *Ann. N. Y. Acad. Sci.* **2012**, *1253*, 16-36; (b) Severi, E.; Hood, D. W.; Thomas, G. H. *Microbiology* **2007**, *153*, 2817-2822; (c) Reuter, G.; Gabius, H. J. *Biol. Chem. Hoppe Seyler.* **1996**, *377*, 325-342.
4. (a) Ress, D. K.; Linhardt, R. J. *Curr. Org. Synth.* **2004**, *1*, 31-46; (b) Haberman, J. M.; Gin, D. Y. *Org. Lett.* **2001**, *3*, 1665-1668; (c) Castro-Palomino, J. C.; Tsvetkov, Y. E.; Schmidt, R. R. *J. Am. Chem. Soc.* **1998**, *120*, 5434-5440; (d) Boons, G-J.; Demchenko, A. *Tetrahedron Lett.* **1998**, *39*, 3065-3068; (e) De Meo, C.; Demchenko, A. V.; Boons, G-J. *J. Org. Chem.* **2001**, *66*, 5490-5497 (f) Yu, C-S.; Niikura, K.; Lin, C-C.; Wong, C-H. *Angew. Chem. Intl. Ed.* **2001**, *40*, 2900-2903 (g) Ando, H.; Koike, Y.; Ishida, H.; Kiso, M. *Tetrahedron Lett*. **2003**, *44*, 6883-6886. (h) Adachi, M.; Tanaka, H.; Takahashi, T. *Synlett* **2004**, 609-614. (i) Ando, H.; Koike, Y.; Koizumi, S.; Ishida, H.; Kiso, M. *Angew. Chem. Int. Ed*. **2005**, *44*, 6759-6763. (j) Tanaka, K.; Goi, T.; Fukase, K. *Synlett* **2005**, 2958-2962. (k) Tanaka, S; Goi, T.; Tanaka, K.; Fukase, K. *J. Carbohydr. Chem.* **2007**, *26*, 369-394. (l) Tanaka, K.; Fujii, Y.; Tokimoto, H.; Mori, Y.; Tanaka, S.; Bao, G.-m.; Siwu, E. R. O.; Nakayabu, A.; Fukase, K. *Chem. Asian J.* **2009**, *4*, 574-580; (m) Tanaka, H.; Nishiura, Y.; Takahashi, T. *J. Am. Chem. Soc.* **2006**, *128*, 7124-7125; (n) Tanaka, H.; Nishiura, Y.; Takahashi, T. *J. Org. Chem.* **2009**, *74*, 4383-4386. ; (o) Crich, D.; Li, W. *J. Org. Chem.* **2007**, *72*, 2387-2391. (p) Hsu, C.-H.; Chu, K.-C.; Lin, Y.-S.; Han, J.-L.; Peng, Y.-S.; Ren, C.-T.; Wu, C.-Y.; Wong, C.-H. *Chem. Eur J.* **2010**, *16*, 1754-1760.

5. Boons, G-J.; Demchenko, A. *Chem. Rev.* **2000**, *100*, 4539-4565.
6. (a) Kanie, O.; Kiso, M.; Hasegawa, A. *J. Carbohydr. Chem.* **1988**, *7*, 501-506. (b) Hasegawa, A.; Ohki, H.; Nagahama, T.; Ishida, H.; Kiso, M. *Carbohydr. Res.* **1991**, *212*, 277-281.
7. (a) Marra, A.; Sinay, P. *Carbohydr. Res.* **1990**, *195*, 303-308; (b) Birberg, W.; Lönn, H. *Tetrahedron Lett.* **1991**, *32*, 7453-7456; (c) Martichonok, V.; Whitesides, G. M. *J. Org. Chem.* **1996**, *61*, 1702-1706.
8. Hanashima, S.; Akai, S.; Sato, K. *Tetrahedron Lett.* **2008**, *49*, 5111-5114.
9. Wang, Y.; Ye, X-S. *Tetrahedron Lett.* **2009**, *50*, 3823-3826.
10. Cai, S.; Yu, B. *Org. Lett.* **2003**, *5*, 3827-3830.
11. Uchinashi, Y.; Nagasaki, M.; Zhou, J.; Tanaka, K.; Fukase, K.; *Org. Biomol. Chem.* **2011**, *9*, 7243-7248.
12. Meijer, A.; Ellervick, U. *J. Org. Chem.* **2004**, *69*, 6249-6256.

Rapid Separation Methods

As improved synthesis techniques get discovered and more efficient instruments become available for use, it is not surprising that more challenging compounds, as well as transformations, have caught the interest of many. Aside from devising a synthetic route that will afford the product in the shortest reaction time and least amount of by-products, the ease of purification is also a vital concern. In some cases, the purification technique to be used might even dictate the method by which a certain chemical transformation will be carried out. In carbohydrate chemistry, where the synthesis of complex substances require purification at almost each step, the incorporation of fast separation techniques in the synthetic route itself has become a very important tool. Examples of these include solid-phase synthesis, and phase separation using different tagging groups.

Solid-Phase Strategy

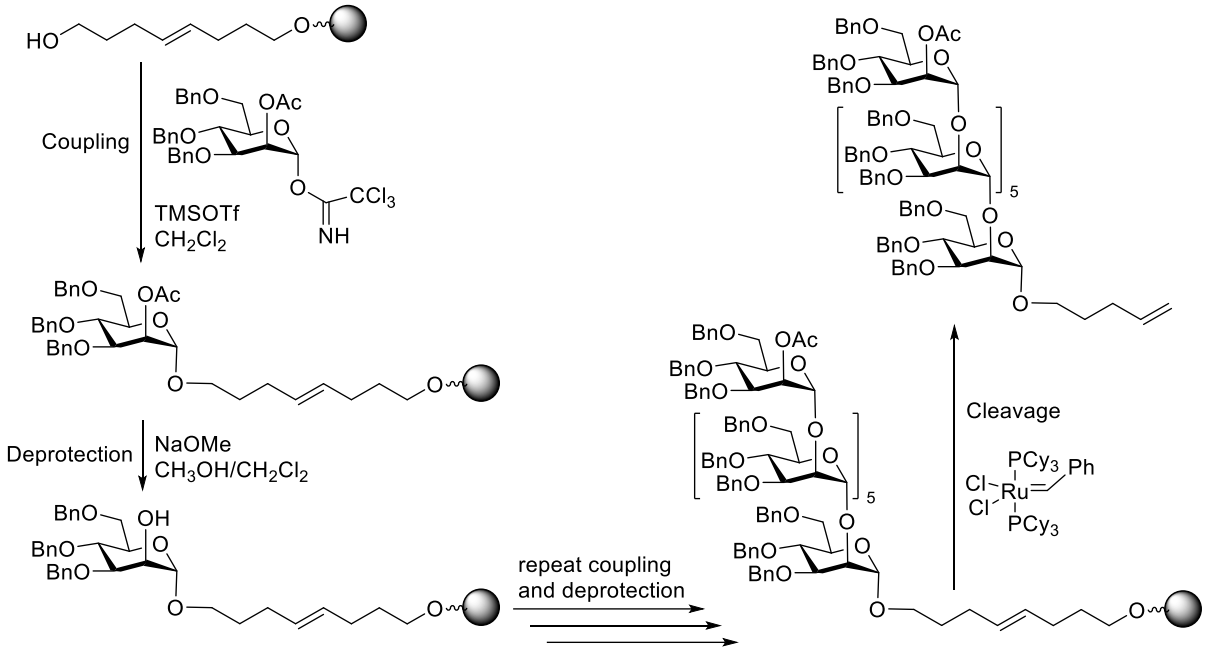
This strategy was initially developed for peptides until further improvements led to its use in the synthesis of small organic molecules and eventual automation. Its application in oligosaccharide synthesis started with Frechet's use of a poly [*p*-(1-propen-3-ol-1-yl) styrene solid support in the synthesis of di- and tri-saccharides. In solid-phase oligosaccharide synthesis (SPOS), reactions are done on resins loaded with appropriate linkers. After coupling the first building block to the linker, a temporary protecting group may be removed followed by a repetition of the coupling until the desired length or branched structure is achieved. This usually ends in the cleavage of the oligosaccharide from the resin and global deprotection making the number of purification steps relatively less in comparison with solution phase synthesis. Since large excesses of reagents is required to drive the reaction to completion, the characteristics of the linker and resin itself play crucial roles in this kind of synthesis. [1]

Most of the solid supports (resins) in SPOS make use of polymers, such as divinyl benzene-polystyrene beads, cross-linked with derivatized polyethylene glycol (PEG), polytetrahydrofuran (PTHF), or other materials to suit a wide range of conditions. The choice of resin often takes into account several factors such as swelling, inertness and capability to withstand mechanical stress. Resins must be able to swell in the common solvents used for synthesis to allow room for the diffusion of the reagents, but inert enough not to be chemically modified by the acids and bases used in the reaction. As a resin swell in a particular solvent, it lends itself to greater site accessibility and functionalization, thus, using a solvent in which the resin does not swell may result in diminished reaction rates. A swelling between 2.0-4.0 mL/g indicates a moderate solvent for a resin, higher than that value is a good solvent while less indicates a poor solvent. On the contrary, the swelling of a resin may not always be proportional to the kinetics of the reaction.[1,2]

Resins used for SPOS can be generally classified into: insoluble such as polystyrene (PS), controlled-pore glass (CPG), and magnetic particles; and soluble such as MPEG, hyperbranched soluble resins, and ionic liquids. Among the insoluble resins, PS resins have been one of the first few to be utilized in both peptide and oligosaccharide syntheses.[3] This includes Merrifield's resin, developed by Robert Bruce Merrifield in the 1960s,

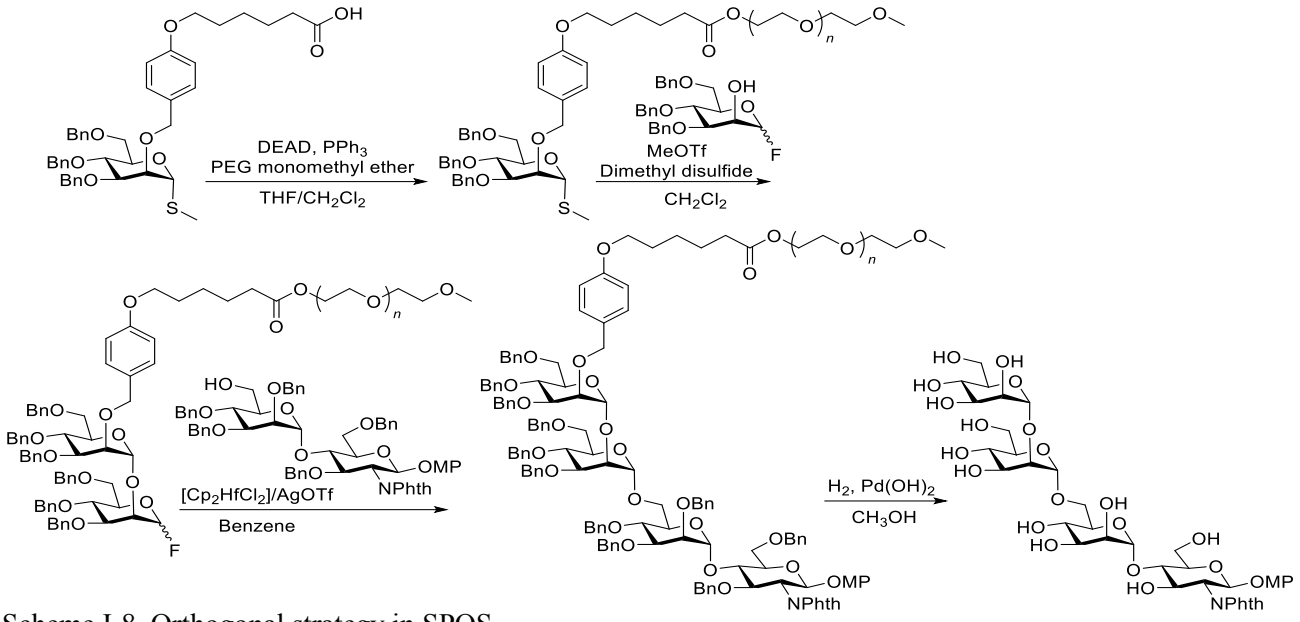
which is composed of PS cross-linked with divinylbenzene (DVB) and is compatible with dimethylformamide (DMF) or dichloromethane (DCM) as solvents.[4] Though PS resins are commonly used due to its compatibility with a wide range of conditions, low cost and high-loading property,[1c] these don't work well with polar solvents unless grafted with hydrophilic poly(ethylene glycol) (PEG) onto the polymer backbone. Examples of this kind of resins include TentaGel and ArgoGels with the former being compatible with polar solvents but suffers from problems of lower resin substitution for which the latter is an alternative. Non-grafted PS-MPEG resins with resin substitutions and solvent compatibilities superior to Merrifield, TentaGel and ArgoGels have also been used.[3,4] As opposed to PS resins that must be allowed to swell in solvents prior to reaction, CPG beads are non-swelling solid supports more commonly used for oligonucleotide synthesis. Its advantages include fast washing, lower acid promoter requirement, and no repetition of coupling reactions.[5] However, CPG beads are susceptible to fractures and are not compatible with silyl ethers. Magnetic particles, on the other hand, have high loading capacities without the need for swelling and is useful in capillary reactors. As for soluble resins, these are not as widely used as insoluble resins, with the exception of soluble MPEG, but can be tailored to exhibit properties that beneficial for very specific investigations. For instance, dendrimers have been used to investigate protein-antigen interactions, although high loading yield is offset by high cost of production. Alternative to that in oligosaccharide synthesis is the use of a hyperbranched polyester which provides the same high loading yield at minimized costs.[3,6]

Aside from the choice of resin, the strategy by which the oligosaccharide will be synthesized on the solid supports is also considered. In general, there are two approaches in SPOS: reducing to non-reducing end, and non-reducing end to reducing end. In the first strategy, the acceptor is the one bound to the solid supports while the glycosyl donor and promoter are in the solution. Deprotection of a certain protecting group is usually done after the initial product is obtained to generate a new acceptor. This can be done if the reactivity of both substrates are enough for the reaction to proceed to completion.[7] Seeberger applied this strategy in the automated synthesis of oligosaccharides, such as α-(1→2) mannoside decamer, and phytoalexin elicitor β-glucans. The synthesis of α-(1→2) mannoside was done by attaching 4-octenediol as a linker to a polystyrene resin followed by coupling with the first building block containing a trichloroacetimidate anomeric leaving group. After washing with MeOH/DCM to remove all excess reagents and by-products, the acetyl protecting group at C2 was cleaved using NaOMe in MeOH/DCM mixed solvent to generate a new acceptor followed by coupling with the second building block, and then washing. This cycle was repeated until the desired length of oligosaccharide was obtained. Cleavage from the resin was done by olefin cross metathesis reaction. Comparison of the results of automated and manual solid phase syntheses of α-(1→2) mannoside heptamer revealed that the automated synthesis is more efficient in terms of reaction time and yield (Scheme I-7). Aside from utilizing trichloroacetimidate donors, Seeberger also introduced glycosyl phosphates as donors in both solid and solution phase syntheses.[8]



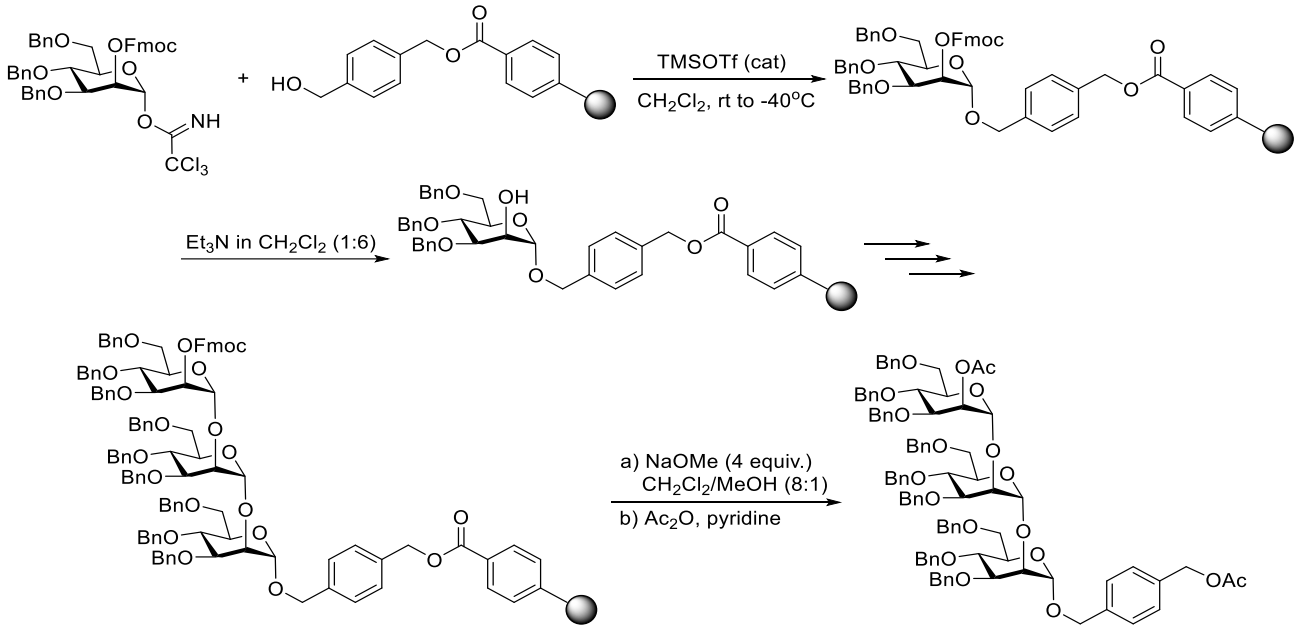
Scheme I-7. Automated solid phase synthesis using polystyrene resin.

The second strategy, on the other hand, proceeds with the donor bound to the polymer while the acceptor and promoter are in solution. In this approach, the by-products can also accumulate in the polymer together with the desired product. One solution to this problem, suggested by Ogawa, is the use of an orthogonal approach in glycosylation wherein a tag was attached at the anomeric position of the reducing end of the oligosaccharide followed by purification using reverse-phase silica gel chromatography. This enabled the synthesis of a fragment that corresponds to a partial protected structure of glycosylphosphatidylinositol (GPI) anchor (Scheme I-8).[7]



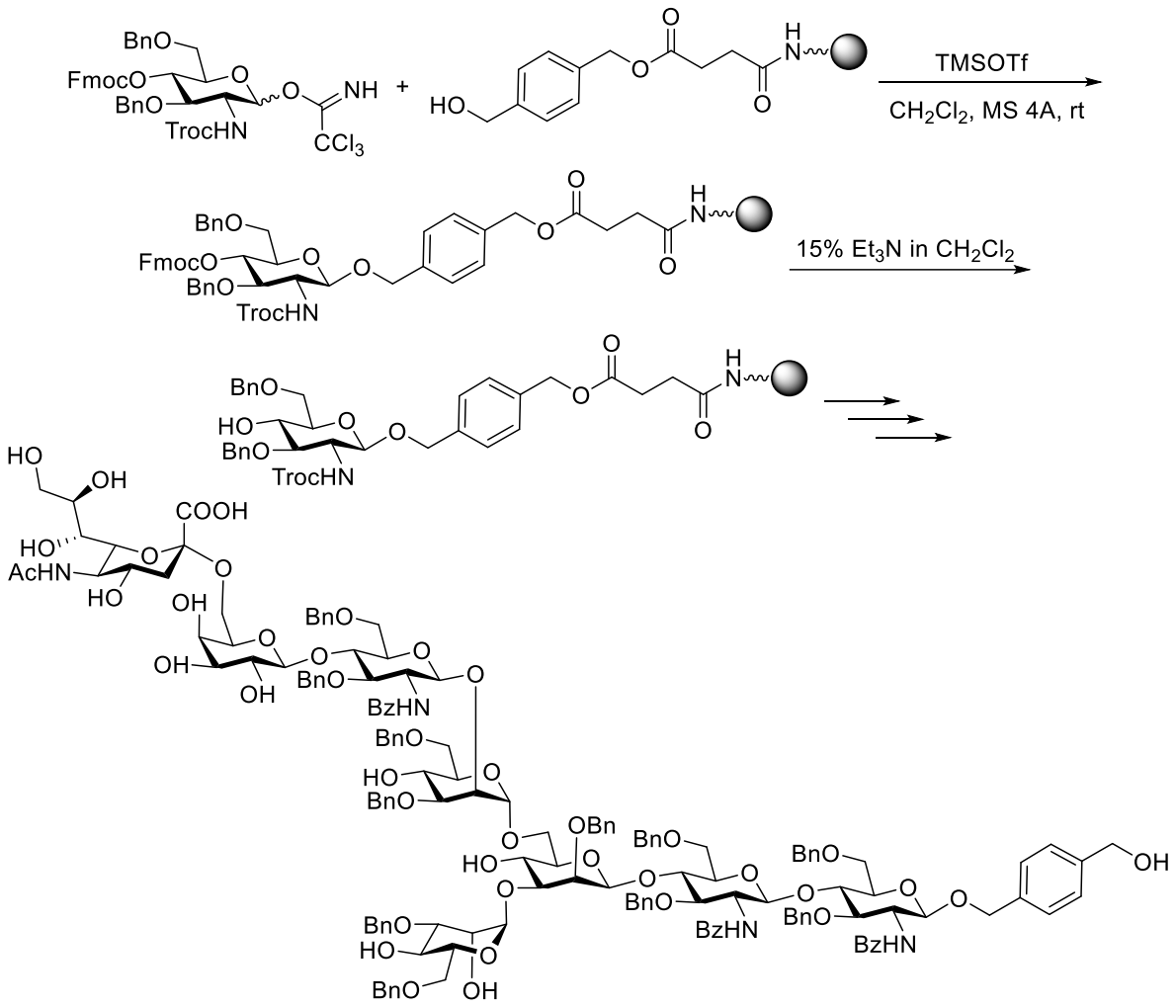
Scheme I-8. Orthogonal strategy in SPOS.

Aside from *O*-glycans, *N*-glycans have also been synthesized using resins. Schmidt reported the synthesis of a small library of *N*-glycans on Merrifield resin using various ester protecting groups which are orthogonally cleavable at different stages (Scheme I-9). These include a benzoate linker, Fmoc and phenoxyacetyl groups as temporary protecting groups cleaved during elongation of the glycan chain, and benzyl group as a permanent protecting group and spacer. Trichloroacetimidates activated by catalytic amounts of TMSOTf were used as donors in the syntheses where both branched and linear glycans were produced.[8]



Scheme I-9. Solid-phase synthesis of *N*-glycans.

A previous work in our group also involved the synthesis of sialic acid-containing *N*-glycan on JandaJel resin as solid support. In this case, trifluoroacetimidate donors activated by TMSOTf were used in the glycosylation reactions where an octasaccharide containing sialic acid was synthesized in 27% overall yield (based from the introduction of the first fragment onto the resin) (Scheme I-10).[9]



Scheme I-10. Solid-phase synthesis of sialic acid-containing *N*-glycans.

Recent developments in carbohydrate chemistry not only saw the increased applications of solid supports in the synthesis of *O*-glycans, but that of *N*-glycans as well with the use of a variety of linkers. These linkers facilitate the attachment of the building blocks to the solid support. An example of which is 4-octanediol that was introduced by Seeberger,[10] or 4-Azido-3-chlorobenzyl, which was previously reported by our group (see section on Phase Tags).

In the present work, several linkers were investigated in an attempt to load asparagine-linked glucosamine into solid supports as preliminary work for the synthesis of *N*-glycans via solid phase. One of candidates that

the author found is allyl ester-type linker. Although the solid-phase synthesis of *N*-glycans using this linker is yet to be investigated, this linker is expected to apply for solid-phase or phase-tag synthesis of various *N*-glycan partial structures.

References:

1. (a) Santini, R.; Griffith, M. C.; Qi, M. *Tetrahedron Lett*. **1998**, *39*, 8951-8954; (b) Frechet, J. M.; Schuerch, C.; *J. Am. Chem. Soc*. **1971**, *93*, 492-496; (c) Weishaupt, M.; Eller, S.; Seeberger, P. *Methods Enzymol*. **2010**, *478*, 463-484.
2. (a) Toy, P.; Janda, K. *Tetrahedron Lett*. **1999**, *40*, 6329-6332; (b) Vaino, A. R.; Goodin, D. B.; Janda, K. D. *J. Comb. Chem*. **2000**, *2*, 330-336.
3. Seeberger, P. H.; Wippo, H. Oligosaccharides. In *The Power of Functional Resins in Organic Synthesis*; Tulla-Puche, J.; Albericio, F., Ed.; Wiley-VCH: Weinheim, Germany, 2008; pp 585-611.
4. (a) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 21492154; (b) Alesso, S. M.; Yu, Z.; Pears, D.; Worthington, P. A.; Luke, R. W. A.; Bradley, M. *Tetrahedron* 2**003**, *59*, 7163-7169.
5. Adinolfi, M.; Barone, G.; De Napoli, L.; Iadonisi, A.; Piccialli, G. *Tetrahedron Lett.* **1998**, *39*, 1953-1956.
6. Kantchev, A. B.; Parquette, J. R. *Tetrahedron Lett*. **1999**, *40*, 8049-8053.
7. (a) Ito, Y.; Kanie, O.; Ogawa, T. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2510-2512; (b) Danishefsky, S. J.; Bilodeau, M. T. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1380-1419.
8. Jonke, S.; Liu, K-g.; Schmidt, R. R. *Chem. Eur. J.* **2006**, *12*, 1274-1290.
9. Tanaka, K.; Fujii, Y.; Tokimoto, H.; Mori, Y.; Tanaka, S.; Bao, G-m.; Siwu, E. R. O.; Nakayabu, A.; Fukase, K. *Chem. Asian J.* **2009**, *4*, 574-580.
10. (a) Plante, O. J.; Palmacci, E. R.; Seeberger, P. H. *Science* **2001**, *291*, 1523-1527; (b) Andrade, R. B.; Plante, O. J.; Melean, L. G.; Seeberger, P. H. *Org. Lett.* **1999**, *1*, 1811-1814; (c) Plante, O. J.; Andrade, R. B.; Seeberger, P. H. *Org. Lett.* **1999**, *1*, 211-214.

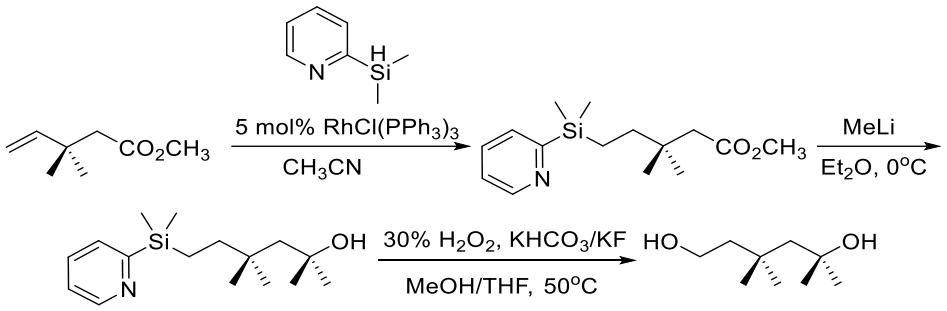
Phase Tagging

The application of solid phase strategy in the synthesis of oligosaccharides facilitated the removal of tedious purification steps that also require much amount of solvents. However, reactions that require very strict control of the temperature, especially very high (>120°C) or very low (<-50°C), are quite challenging since the heterogeneity of the solid phase system may cause the temperature of the reaction mixture to vary a few degrees from the temperature that was set in the instrument. Reaction rates using resins are also, in general, lower than their solution phase counterparts.[1] Thus, a reaction with poor yields in solution may not even proceed if done on solid phase. Another limitation of solid phase synthesis is the monitoring of the reaction progress because normal thin layer chromatography cannot be employed. In this case, reactions have to be carried out first in solution and the conditions optimized before it can be done using resins. Because of these, other kinds of tagging strategies have emerged, one of which is called phase tag strategy.

Tags are substances or molecular fragments covalently bonded to the desired compound for purposes of identification or easier purification. Those that alter a compound's affinity in phase separation techniques such as solid/liquid and liquid/liquid extractions are called phase tags. As tags can be attached to a substrate before the series of transformations are done, or after the desired compound has been synthesized and prior to final purification, these must be inert under the reaction conditions to be utilized. Their installation or removal must also not produce drastic alterations in the reactivity of the target compounds.[2]

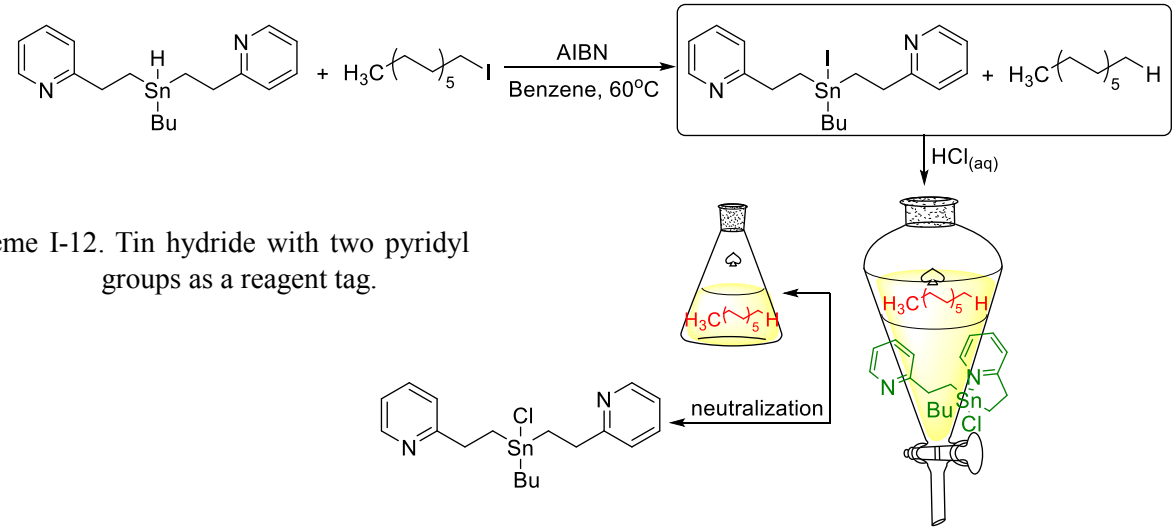
Phase tags can be classified based on their attachment or the nature of their preferred phase. Tags classified based on their attachments can be substrate tags, reagent tags or catalyst tags. Substrate tags are connected prior to the reaction sequence and then cleaved after the desired compound has been synthesized. Reagent tags, on the other hand, can be introduced after the reaction to recover the excess reagents. Catalyst tags are used to allow the easy separation and recovery of a certain catalyst after one particular reaction.[2] In some cases, tagged catalysts can be reused after washing or reactivation.

One example of a substrate tag is 2-pyridyldimethylsilyl (2-PyMeSi) which is capable of phase switching in acid/base extractions. Tags containing pyridyl groups are called masked phase tags which can be activated by protonation of the pyridine ring.[3] In acidic extraction, 2-PyMeSi-tagged compounds are present in the aqueous layer. When the aqueous phase is neutralized, the tagged compounds then transfer to the organic layer. Attachment of the tag to the substrate, done by Yoshida, was achieved by $RhCl(PPh)_3$-catalyzed hydrosilylation and cleaved at the end using Tamao-Fleming oxidation. Multi-step synthesis was done with 2-PyMeSi not only acting as a tag, but also a directing group via pre-coordination of pyridyl with the metal catalyst. In this case, acid-base extraction performed after each reaction prior to tag removal afforded relatively pure products which were directly used in succeeding steps (Scheme I-11).[4]



Scheme I-11. 2-PyMeSi as acid/base phase tag.

Two pyridyl groups attached to Tin hydride can also be used as a reagent tag in the reduction of organic iodides and bromides with Bu_3SnH/AIBN via a radical reaction. After acid/base extraction, the tin halide can be completely removed which is not the case if there is only one pyridyl group attached to tin hydride (Scheme I-12).[2,5]



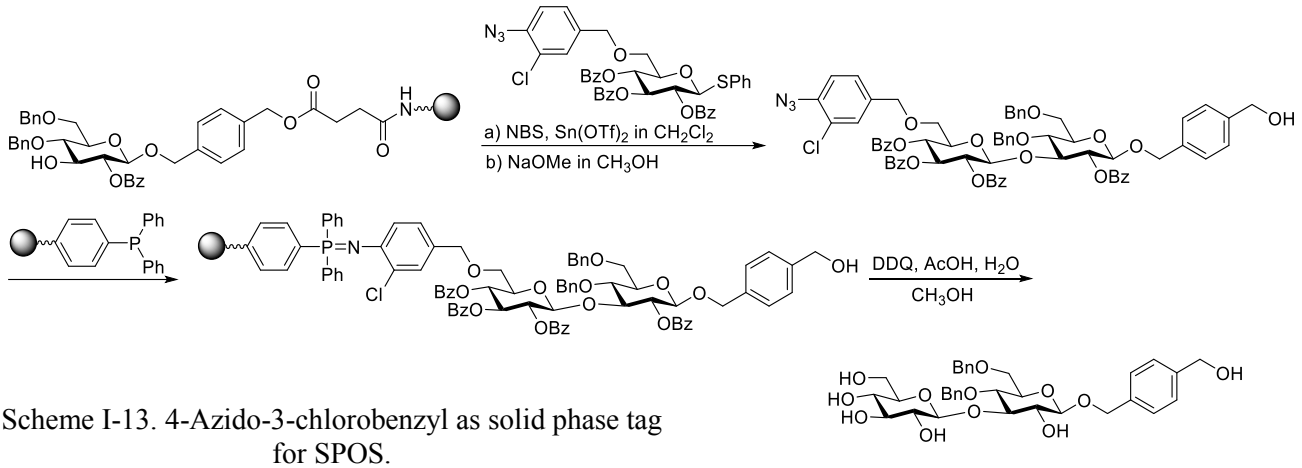
Scheme I-12. Tin hydride with two pyridyl groups as a reagent tag.

Some of the more common examples of catalyst tags, on the other hand, include fluorous biphase catalysts by Horváth and Gladysz. Highly fluorinated alkane, ether, and tertiary amines form bilayers with organic solvents. When metal complexes such as that of rhodium are attached with fluoroalkyl groups, these become miscible with fluorinated solvents facilitating their easy separation from the rest of the reaction mixture. An example of this is $ClRh[PCH_2CH_2(CF_2)_5CF_3]_3$ which was found to be an effective catalyst for hydroborations involving styrene and catecholborane at ~45°C even without fluorous solvents. The products were extracted using tetrahydrofuran (THF) while the recovered catalyst can be recycled.[6]

Another classification of phase tags is the one based on the nature of their preferred phase, namely: solid, aqueous, fluorous, ionic liquid, and supercritical fluid phases. A tag for solid phase can be divided into two: for

precipitation or for resin capture. The first type is soluble during the reaction, and when the product has formed, the tag can be isomerized to induce precipitation. This can then be separated using simple filtration methods. The second type acts as a linker that can bind the tagged molecule to the solid support (resin) via covalent or ionic interactions. These are cleaved from the final product at the end of the reaction sequence.[2]

An example of the second type of tag for solid phase as reported by our group involves the use of 4-azido-3-chlorobenzyl (ClAzb) both as a tag and a protecting group in SPOS. Synthesis was done by attaching a 3-OH glycosyl acceptor to an ArgoPore-NH_2 resin via a benzyl ester-type linker followed by glycosylation with a thioglycoside having ClAzb as a protecting group in the 6-position. After the reaction, the disaccharide was released from the ArgoPore-NH_2 resin by cleavage of the benzyl ester linker with sodium methoxide in methanol (NaOMe/MeOH). The products obtained were then captured by a commercially available triphenylphosphine-(polyethyleneglycol-polystyrene-copolymer) resin via the interaction of the azide group in the disaccharide and the phosphine in the resin. Subsequently, the disaccharide was released from the resin by treatment with DDQ then treated with ion-exchange resins to remove some by-products (Scheme I-13).[7]

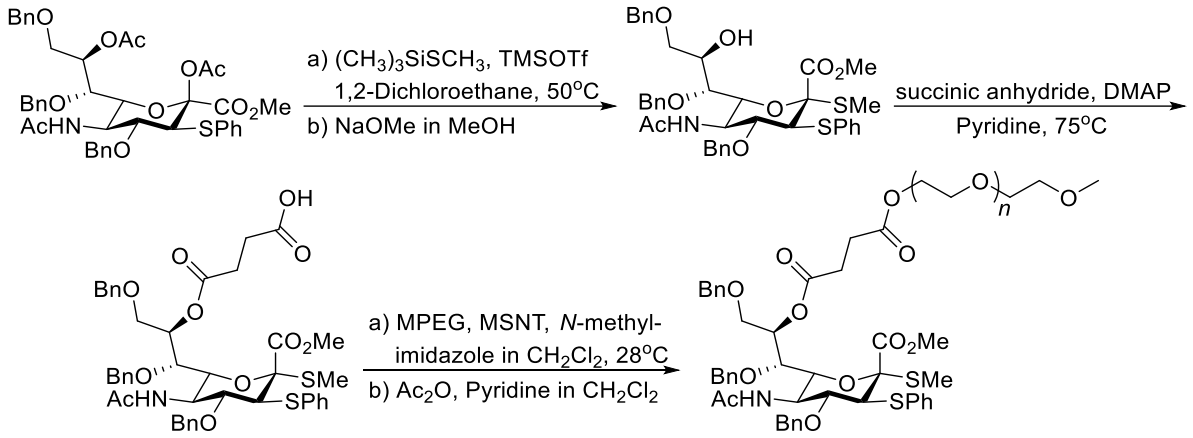


Scheme I-13. 4-Azido-3-chlorobenzyl as solid phase tag for SPOS.

As for phase tags for the aqueous phase, which commonly involve acidic and basic extractions, the 2-$PyMe_2Si$ substrate/reagent tag previously discussed provides a good example. Tags for fluorous phase such as functionalized perfluoroalkyl groups, on the other hand, capitalize on the ability of fluorous solvents to mix with organic solvents at elevated temperature where reactions are carried out. When the reaction is finished and the reaction mixture eventually cooled to room temperature, these fluorous solvents gradually become immiscible with organic solvents forming biphasic layers. In this case, at high temperatures, the substrate and the tagged molecule are in a monophasic system facilitating the reaction. After the mixture is cooled, the tagged molecules remain in the fluorous solvent while the product is in the organic solvent which, in turn, facilitates easy purification.

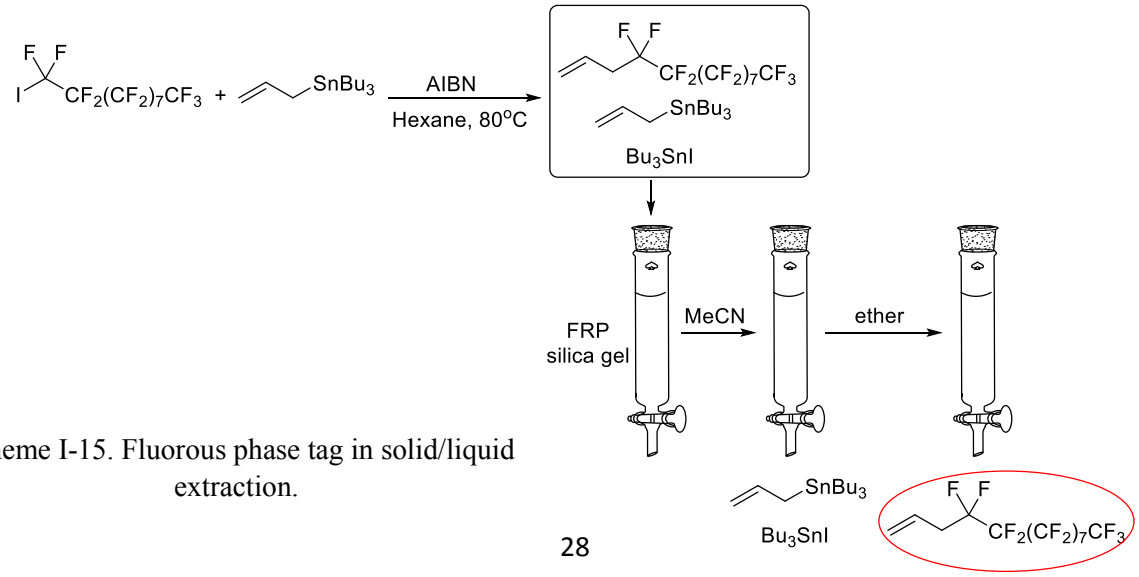
Thioglycosides have also been attached to polymer supports such as polyethylene glycol monomethyl ether (MPEG) in an orthogonal glycosylation method. In the strategy by Ogawa, thiophenyl was used as an auxiliary

in C3 to control stereoselectivity while the polymer was attached via a succinate at C8. Activation using DMTST gave moderate yields even with the 2→3 sialylation (Scheme I-14). In addition, the use of MPEG, a soluble resin for solid phase synthesis, allowed easy purification of the product by precipitation from *t*-butyl methyl ether.[8]



Scheme I-14. Sialic acid glycosyl donor using polymer supports. (DMAP: 4-Dimethylaminopyridine, MSNT: 1-(2-Mesitylenesulfonyl)-3-nitro-1H-1,2,4-triazole)

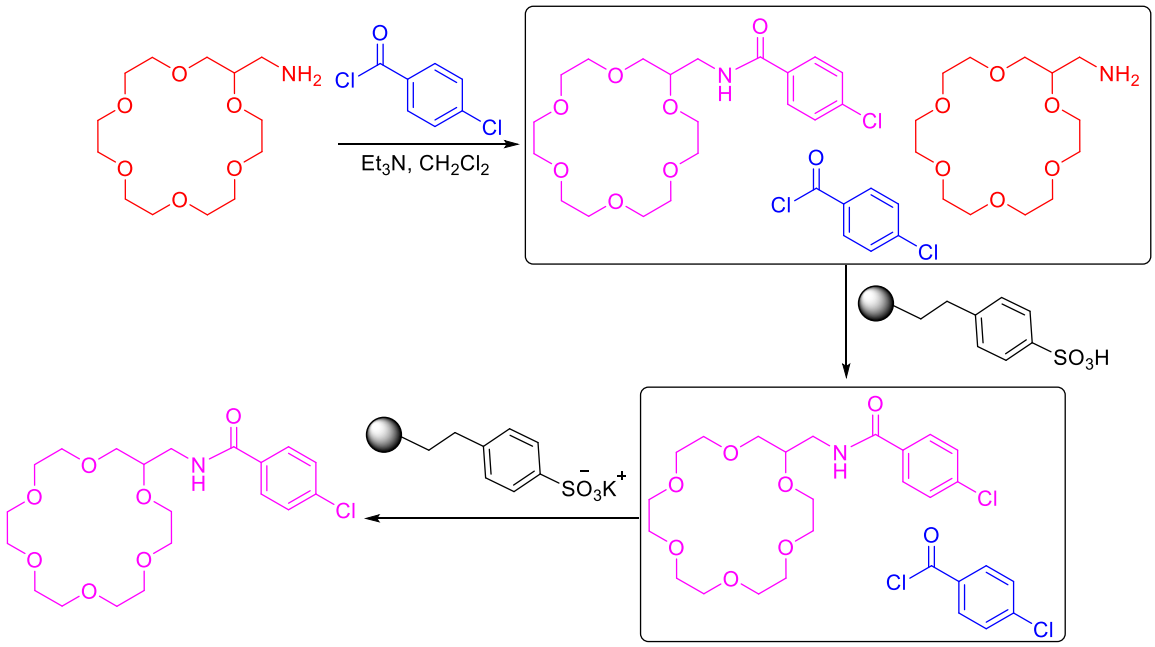
Curran also utilized fluorous tags not only in liquid/liquid extractions but in also in solid/liquid extractions with the help of fluorous reverse-phase (FRP) silica gel columns such as Fluofix. In the reaction of perfluorodecyl iodide with allyltributyltin the product 3-(perfluorodecyl)prop-1-ene was purified using a short FRP silica gel column that was washed with CH_3CN. The crude reaction mixture was loaded onto the column then washed with CH_3CN to remove the excess reagents followed by washing with ether to elute the product (Scheme I-15).[2,9]



Scheme I-15. Fluorous phase tag in solid/liquid extraction.

Ligands of transition metal complexes can also be replaced by fluorous tags. An example of which is $Rh/P[CH_2CH_2(CF_2)_5CF_3]_3$, which was found to be more stable than Rh/PPh_3 in the hydroformylation of ethylene.[10] Others include fluorous-tagged Mn-porphyrins,[11] or Co complexes of perfluoroalkylated tetraarylporphyrins and phthalocyanins.[12] Palladium complexes containing fluorous dialkyl sulfides have also been reported in Suzuki coupling reactions.[13] The use fluorous tags in enzymatic reactions have also been reported by Beckman synthesized nicotinamide adenine dinucleotide (NAD) with a fluorinated polymer tail and used it as a soluble coenzyme in reactions done in methoxynonafluorobutane (HFE).[14]

In a report by Lepore, aminomethyl-18-crown-6 ether was reacted with 4-chlorobenzoylchloride only until 50% of the product has formed and some unreacted substrates still remained. The reaction mixture was then allowed to pass through a silica-supported ethylbenzenesulfonic acid (SCX) ion-exchange column previously treated with AcOH in MeOH. After rinsing with methanol, the product with another unreacted starting material was obtained. This mixture was allowed to pass through another SCX column previously treated with saturated K_2CO_3 in MeOH. Washing with MeOH only gave the unreacted starting material while washing with saturated K_2CO_3 in MeOH gave the crown ether-containing product in high purity (Scheme I-16).[15]



Scheme I-16. 18-Crown-6 ether as a phase tag.

The author's laboratory had also previously investigated another kind of phase tag strategy is called 'synthesis based on affinity separation'. This strategy works by allowing the reaction mixture to pass through a resin having a functional group that can recognize the tag of the compound thereby retaining it in the resin until desorbed by suitable solvents. In this way, the rest of the reaction mixture can be eluted by washing with a different solvent, leaving the pure tagged compound (Figure III-3).[1,16]

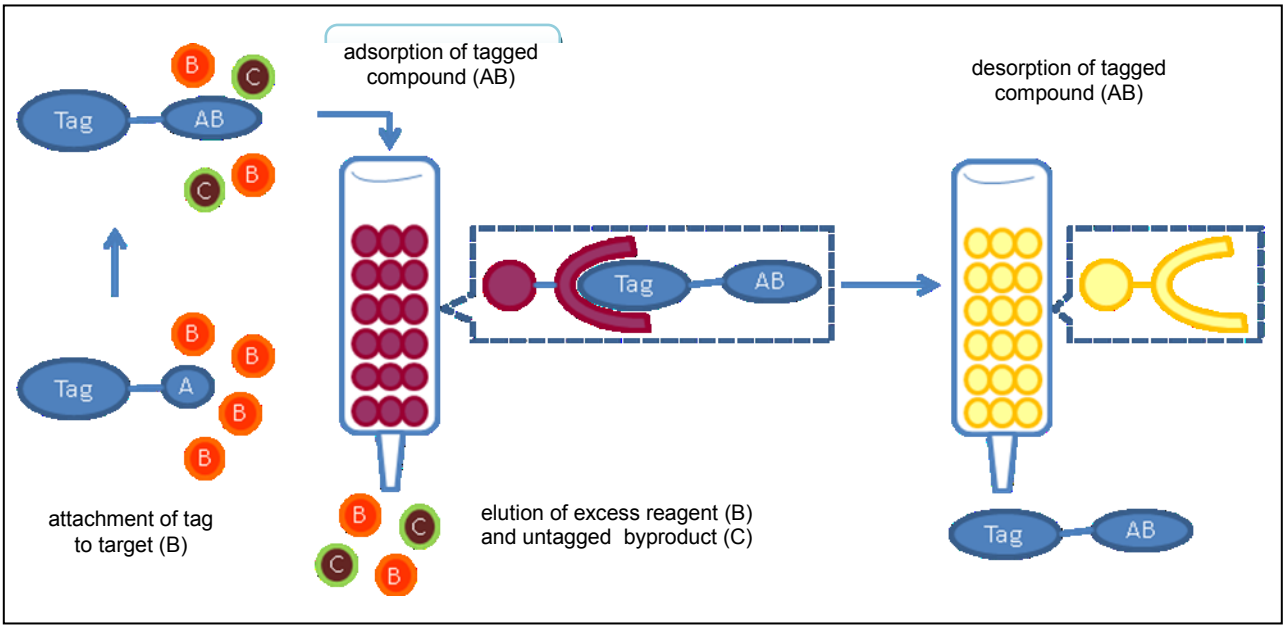


Figure III-3. Synthesis based on Affinity Separation.

In this kind of phase tagging strategy, the interaction of 32-crown-10 ether, which served as a tag, with aminomethylated polystyrene resin was utilized for the faster and easier purification of the synthesized peptides and heterocycles.[1] After a reaction, the crude mixture was allowed to pass through a column containing ArgoPore-NH_2 beads previously treated with TFA. All other compounds, except the one connected to the crown ether tag, are eluted using a nonpolar solvent such as dichloromethane. On the other hand, the crown ether-tagged compound is eluted using a more polar solvent such as DCM:MeOH (1:1). An improvement of that strategy was published subsequently, where instead of the crown ether tag, a podand tag was utilized.[16c] The latter was easier to synthesize while still retaining high affinity toward the ammonium ions in the resin facilitating the efficient synthesis of several oligosaccharides (Figure III-4).

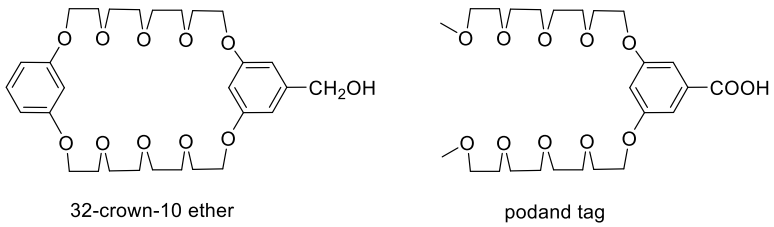


Figure III-4. Affinity Tags

In this current work, Triton X-100, a commercially available polyethylene glycol-containing compound, was used as an affinity tag in the synthesis of sialic acid-containing oligosaccharides. As compared with the affinity tags previously published, Triton can be attached directly to the substrate without prior modification, reducing the time needed to complete the synthetic work. Here, the galactoside acceptor was attached to the triton tag followed by sialylation employing the conditions for donor activation with $ICl/In(OTf)_3$. In normal reactions without employing tags, purification of the disaccharide is difficult because the polarities of the product and donor derivatives are similar on silica-gel. However, with the use of Triton as affinity tag, the product was readily separated from the donor derivatives. Details are discussed in Chapter III.

References:

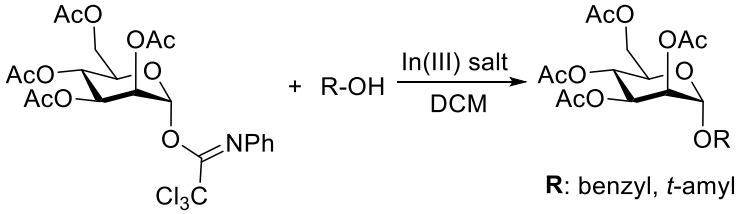
1. Zhang, S.-Q.; Fukase, K.; Kusumoto, S. *Tetrahedron Lett*. **1999**, *40*, 7479-7483.
2. Yoshida, J.; Itami, K. *Chem. Rev.* **2002**, *102*, 3693-3716.
3. El Bakkari, M.; Vincent, J-M. *QSAR Comb. Sci.* **2006**, *25*, 689-696.
4. Yoshida, J.; Itami, K.; Mitsudo, K.; Suga, S. *Tetrahedron Lett.* **1999**, *40*, 3403-3406.
5. Suga, S.; Manabe, T.; Yoshida, J. *Chem. Commun.* **1999**, 1237-1238.
6. Juliette, J. J. J.; Rutherford, D.; Horváth, I. T.; Gladysz, J. A. *J. Am. Chem. Soc.* **1999**, *121*, 2696-2704.
7. Egusa, K.; Kusumoto, S.; Fukase, K. *Synlett* **2001**, *6*, 777-780.
8. Kononov, L. O.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1997**, *38*, 1599-1602.
9. (a) Curran, D. P. *Angew. Chem. Int. Ed.* **1998**, *37*, 1174-1196; (b) Luo, Z.; Zhang, O.; Oderaotoshi, Y.; Curran, D. P. *Science* **2001**, *291*, 1766-1769; (c) Zhang, W. *Curr. Opin. Drug Discov. Devel.* **2004**, *7*, 784-797; (c) Curran, D. P. *Med. Res. Rev.* **1999**, *19*, 432-438.
10. Horvàth, I. T.; Kiss, G.; Cook, R. A.; Bond, J. E.; Stevens, P. A.; Ràbai, J.; Mozeleski, E. J. *J. Am. Chem. Soc.* **1998**, *120*, 3133-3143.
11. Pozzi, G.; Banfi, S.; Manfredi, A.; Montanari, F.; Quici, S. *Tetrahedron* **1996**, *52*, 11879-11888.
12. Colonna, S.; Gaggero, N.; Montanari, F.; Pozzi, G.; Quici, S. *Eur. J. Org. Chem.* **2001**, *2001*, 181-186.
13. Rocaboy, C.; Gladysz, J. A. *Tetrahedron* **2002**, *58*, 4007-4014.
14. Panza, J. L.; Russell, A. J.; Beckman, E. J. *Tetrahedron* **2002**, *58*, 4091-4104.
15. Lepore, S. D. *Tetrahedron Lett.* **2001**, *42*, 6437-6439.
16. (a) Zhang, S-Q.; Fukase, K.; Izumi, M.; Fukase, Y.; Kusumoto, S. *Synlett* **2001**, *5*, 590-596; (b) Fukase, K.; Zhang, S.-Q.; Fukase, Y.; Umesako, N.; Kusumoto, S. In *New Discoveries in Agrochemicals*; Clark, J. M.; Ohkawa, H., Eds; ACS Symposium Series 892, American Chemical Society: Washington, DC, 2005; pp 87-98; (c) Fukase, K.; Takashina, M.; Hori, Y.; Tanaka, D.; Tanaka, K.; Kusumoto, S. *Synlett* **2005**, *15*, 2342-2346.

Chapter II Effective α-Sialylation Using IX/In(OTf)$_3$ Promoter System

Introduction

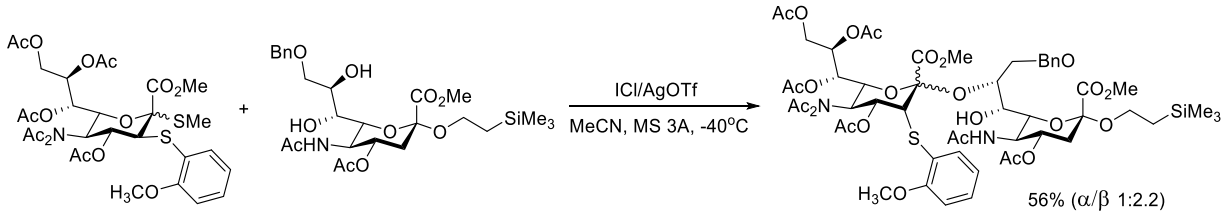
With the demand for carbohydrate-based compounds as scaffolds for drug discovery, research has shifted into less sensitive reagents that can tolerate a wide range of reactions with very little toxicity and/or close to neutral conditions. Glycosylation, one of the most important reactions in carbohydrate chemistry, saw a transition in the Lewis acids used as promoters from AgOTf and $FeCl_3$ to alternatives such as $BF_3 \cdot OEt_2$ and TMSOTf that circumvent the production of heavy metals.[1]

Indium is a non-genotoxic metal commonly used in microelectronics[2] and its salts were found to promote reactions even in aqueous media.[3] $InCl_3$ has been reported to catalyze Ferrier-type rearrangements of per-*O*-acetylglycals with and without the use of additives such as TMSCl resulting in high yields and selectivities even in solvent-free conditions.[4] Aside from $InCl_3$, other indium salts such as $InBr_3$ and $In(OTf)_3$ were also able to promote glycosylation (Scheme II-1) with substrates having acid-labile protecting groups in conditions that were less sensitive to moisture content, extended reaction times and over-heating. In some cases, the catalyst was even recovered without loss of activity.[1,5]



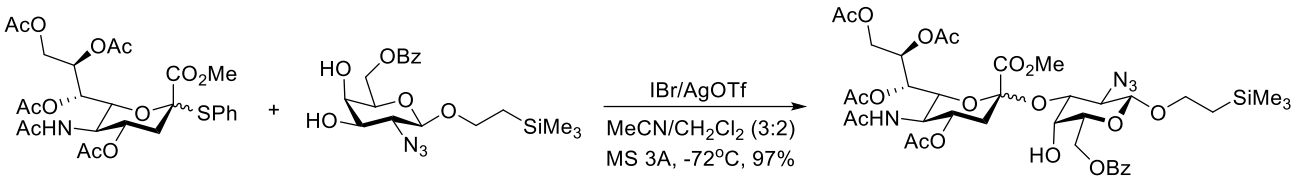
Scheme II-1. Glycosylation using In(III) as promoter.

Iodine interhalogen (IX) compounds, on the other hand, have been reported to activate thioglycosides. The ICl/AgOTf promoter system has been reported in *O*-glycosylation with both donors and acceptors possessing acid- or base-labile protecting groups. No aromatic substitutions of iodine and selective activation of the anomeric methylthio group were observed (Scheme II-2).[6]



Scheme II-2. ICl/AgOTf-promoted thioglycoside activation.

In ICl/AgOTf-promoted reactions, it was reported that the absence of a participating group usually gave low yields. Applying the HSAB theory, donors without participating groups were hard electrophiles which can react with chloride species (hard nucleophile) producing sialic acid halides. Thus, attaching an auxiliary at C3 results in better yields. Another solution to this would be to use an interhalogen species with a much lower reactivity such as IBr since ICl > IBr > I_2 (Scheme II-3). Mechanistic investigations also revealed that the presence of silver ions serve in reducing the concentration of competing halide nucleophiles contributing in the formation of the disaccharide. Furthermore, the use of donors with slower initial reaction rates can decrease glycal formation which is another culprit for low yields in sialylation reactions.[7]



Scheme II-3. IBr/AgOTf-promoted sialylation.

In the reaction pathway proposed by Meijer, the activated complex formed when IX interacts with Ag ions reacts with the thioglycoside **I** forming complex **II** which can collapse into an oxocarbenium ion **III**. The product is then formed from this oxocarbenium ion and a sulfenyl iodide species is generated as a by-product. This sulfenyl iodide species can be activated by another Ag ion to form a sulfonium ion that can also activate another molecule of thioglycoside **I** to form **IV** which, in turn, can also form **III**, thus, completing the cycle (Figure II-1).[8]

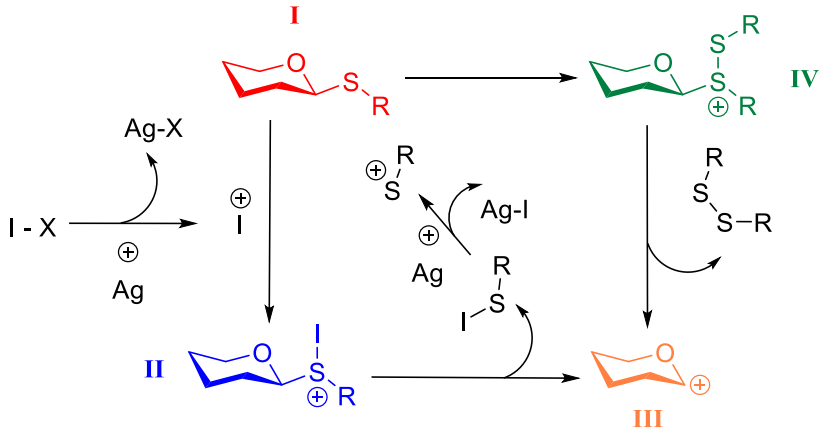


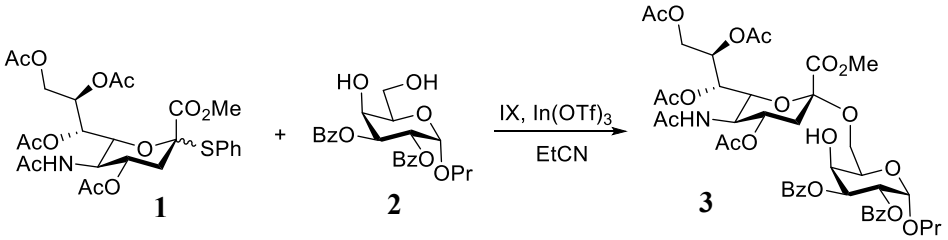
Figure II-1. Proposed IX/AgOTf activation pathway.

To the best of our knowledge, indium (III)-promoted glycosylation involving trifluoroacetimidates as well as thioglycosides with the addition of iodine monochloride, ICl, have not been explored. Herein, we report the use of various indium (III) salts in the glycosylation of both benzyl-protected trifluoroacetimidate donors and acceptors demonstrating the effect of the solvent in the selectivities observed. Furthermore, effective alpha-sialylation was achieved when $In(OTf)_3$ was used together with ICl.

Investigation of IX/$In(OTf)_3$-mediated Sialylation

Because of the unique structure of sialic acids, obtaining high yields and selectivities in sialylation reactions are quite challenging. Even so, the use of nitrile solvents, and conducting the reaction at low temperature are some of the ways by which higher α-selectivity can be achieved. Thus, these were employed in the investigation of $In(OTf)_3$-promoted sialylation. Various oxidants such as NIS, NBS, I_2/NBS, ICl, and IBr were used together with $In(OTf)_3$ in sialylation reactions involving 1 equivalent of the thiophenyl donor and 1.5 equivalent of the α-alkyl galactoside (Table II-1). Two equivalents of $In(OTf)_3$ while 1.2 equivalents of the oxidant was used and the reaction (30 minutes) was monitored by thin layer chromatography (TLC). After the reaction, the unreacted donor together with the disaccharide (same Rf value) were purified by short column chromatography and recorded together as % Recovery while selectivities were calculated using ^{1}H NMR yields.

Table II-1. Investigation of various oxidants in $In(OTf)_3$-promoted sialylation.



Entry	Oxidants	Temperature (°C)	% Recovery	D:α:β
1	NIS	-82	-	NR
2	NBS	-82	-	NR
3	I_2/NBS	-82	-	NR
4	IBr	-80	62	39:54:7
5	ICl	-75	46	16:75:9

D: Unreacted donor

Based from the results, NIS, NBS, and I_2 in combination with $In(OTf)_3$ (entries 1-3) failed to activate the thiophenyl donor resulting in the recovery of the unreacted donor as well as decomposed acceptor. IBr (entry 4) was able to activate the donor within 30 minutes resulting in the formation of the disaccharide. Unfortunately,

much of the unreacted donor was also recovered. On the other hand, less amount of unreacted donor was recovered when ICl (entry 5) was used which may indicate faster activation and formation of the disaccharide. The percentage of recovery was less when ICl was used due to the increased formation of the hydroxylated donor as well as that of glycals. It should be noted that these reactions were not performed in inert atmosphere, thus much moisture was present during the reaction. Even though that was the case, using IBr and ICl together with $In(OTf)_3$ still resulted in the formation of the α-anomeric disaccharide.

A further investigation of IBr and ICl as oxidants was then carried out using the donor, acceptor and $In(OTf)_3$ equivalents given above (Table II-2). Reactions were monitored and deemed finished based on TLC.

Because most of the literature on IBr as oxidant in glycosylation reactions employed IBr in 1 M CH_2Cl_2 solutions, this was also used (entry 1). However, its combination with $In(OTf)_3$ did not result in the desired disaccharide. In entry 2, prolonging the reaction using solid IBr resulted in almost the same selectivity as in Table 1, entry 4, but the recovery was better. In this case, activated molecular sieves 5Å have been added in the reaction mixture. Surprisingly, prolonging the reaction time using solid ICl (entry 3) did not result better recovery or selectivity. When the amount of ICl used was increased and temperature lowered (entry 4), no unreacted donor was recovered and a moderately high α:β selectivity was obtained. Changing the limiting reagent to the acceptor and the excess reagent to the donor in entry 5 did not result in increased % recovery nor selectivity. In this reaction, molecular sieves were not used and the reaction was not done in inert atmosphere. Increased amount of ICl may have activated most of the donor which reacted with the moisture present that lead to more hydroxylated donor. An increased amount of glycals were also observed.

Table II-2. Comparison of IBr and ICl as oxidant.

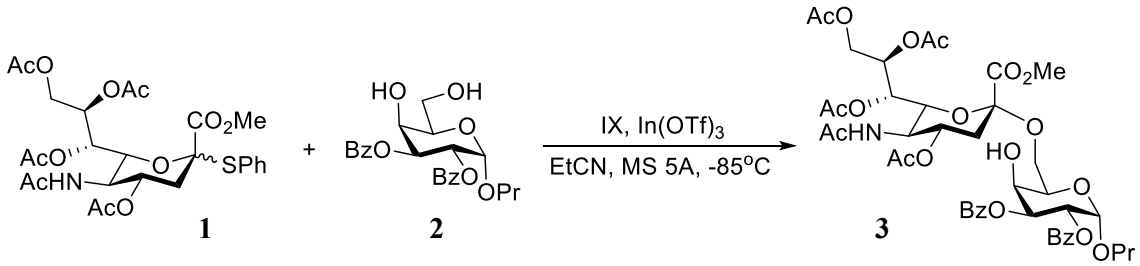
Entry	Oxidants /equivalent	Temperature (°C)	Reaction Time (h)	% Recovery	D:α:β
1	IBr in DCM (1M)/1.2	-80	1.25	69	>99:trace
2[a]	IBr/1.2	-80	3.25	89	40:54:5
3	ICl/1.2	-80	2.0	49	80:20:0
4	ICl/2.9	-85	2.0	43	0:92:8
5[b]	ICl/2.9	-85	overnight	51	46:40:14

a: with molecular sieves 5Å; b: sialic acid (2.5 equivalent), acceptor (1 equivalent); D: Unreacted donor

From these initial findings, it can be seen that addition of activated molecular sieves resulted in the formation of less side products which led to increased % recovery. Increasing the amount of the oxidant also activated all of the donor such that only the disaccharides, hydroxylated donor and glycals were observed. Thus, a further investigation where all these were factored in was done.

In an attempt to increase the % recovery, the limiting reagent was changed to the acceptor while the donor was in excess (Table II-3). In this way, formation of side product and decomposition of the donor will not affect the yield. Activated molecular sieves 5Å was used with all of the reactions done in inert atmosphere and the temperature set at -85°C. The amount of $In(OTf)_3$ used was still set at 2.0 equivalent with the reaction monitored by thin layer chromatography.

Table II-3. Variation in donor equivalent and reaction time.



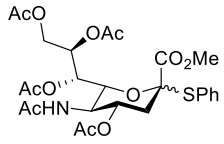
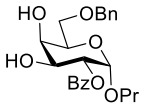
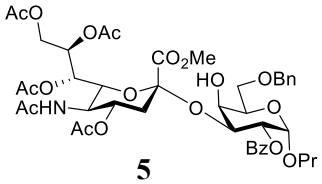
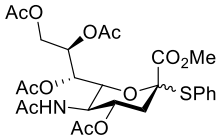
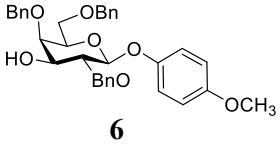
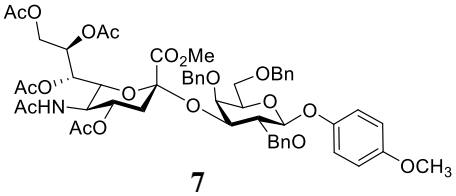
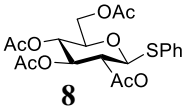
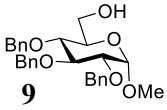
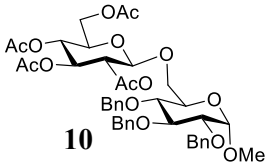
Entry	Oxidants /equivalent	Donor/equivalent	Reaction Time (h)	% Yield (α:β)
1	ICl/2.9	1.5	3	70 (92:8)
2	IBr/2.9	1.5	3	71(90:10)
3	ICl/2.9	2.0	8.5	78 (89:11)
4[a]	ICl/3.9	2.0	24	82 (89:11)

a: ICl equivalent based on sialic acid

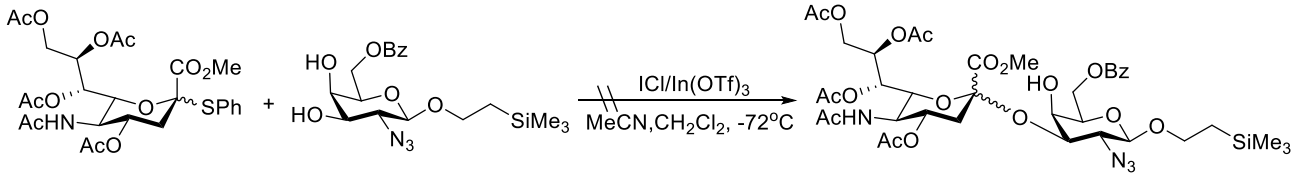
In entry 1, the selectivity obtained when 2.9 equivalents of ICl was used proved to be consistent with the previous result (Table II-2, entry 4), although the yield was better (BRSM calculated at 85%). IBr (entry 2) gave almost the same result as ICl but with slightly lower selectivity. Increasing the amount of donor (entry 3), on the other hand, resulted in slightly lower selectivity and the recovery of unreacted donor even at an extended reaction time. When the amount of oxidant was increased as well (entry 4), the yield was slightly higher and no unreacted donor was recovered though there was no change in selectivity. Thus, entry 1 was considered as the best condition for the sialylation using the $IX/In(OTf)_3$ promoter system.

Table II-4. Application of ICl/In(OTf)$_3$ system to other substrates.

donor + acceptor $\xrightarrow[\text{MS 5A, -85°C}]{\text{ICl, In(OTf)}_3}$ disaccharide

Entry	donor	acceptor	disaccharide	Solvent/% Yield
1	 **1**	 **4**	 **5**	EtCN/complex mixture
2		 **6**	 **7**	EtCN/ 26% (α only) recovered acceptor (79%)
3	 **8**	 **9**	 **10**	CH_2Cl_2, 0°C/ 29% recovered acceptor (30%)

The scope of the new promoter system was tested by applying it in α(2→3) sialylation and β(1→6) *O*-glycosylation (Table II-4). In α(2→3) sialylation, the 3-free hydroxyl acceptor (entry 2) resulted in the formation of the disaccharide in 26% yield as compared with the complex mixture obtained when both the 4 and 3-positions were not protected (entry 1). However, much of the acceptor remained unreacted with some impurities even after short column chromatography. In a similar reaction performed by Meijer[8] using the same donor in combination with another 3,4-free OH acceptor, no disaccharide was detected but the donor was fully consumed. This can be attributed to the interference of nucleophilic Cl^- thereby, forming sialic acid chloride (Scheme II-4).



Scheme II-4. ICl/In(OTf)$_3$ promoter system in α(2→3) sialylation.

On the other hand, the low yield of entry 2 may be attributed to steric hindrance because of the benzyl-protecting group at position 4.

Activation of the acetyl-protected thioglycoside (entry 3) in dichloromethane at 0°C resulted in the disaccharide in 29% yield. Performing this reaction in lower temperature (-85°C) using the same solvent or in EtCN at -85°C only resulted in the formation of the hydroxylated donor and glycals with most of the acceptor recovered.

Summary

We have investigated the conditions for ICl/$In(OTf)_3$-promoted activation of thioglycosides. Though the desired products were obtained even if the reactions were not done in inert atmosphere, the addition of molecular sieves proved beneficial in increasing the yield by suppressed hydroxylated donor formation. It is also important to note that, increasing the amount of ICl activates the donor at a faster rate, thus, its combination with acceptors having lower reactivity favors the formation of side products. Taking this into account, it will be more advantageous to use acceptors with high reactivities and with little steric hindrance from neighboring protecting groups.

References:

1. (a) Mukherjee, D; Ray, P. K.; Chowdhury, U. S. *Tetrahedron* **2001**, *57*, 7701-7704; (b) Mattson, A. L.; Michel, A. K.; Cloninger, M. J. *Carbohydr. Res.* **2012**, *347*, 142-146.
2. Asakura, K.; Satoh, H.; Chiba, M.; Okamoto, M.; Serizawa, K.; Nakano, M.; Omae, K. *J Occup Health* **2009**, *51*, 498-512.
3. Loh, T-P.; Chua, G-L. *Chem. Commun.* **2006**, 2739-2749.
4. (a) Ghosh, R.; De, D.; Shown, B.; Maiti, S. B. *Carbohydr. Res.* **1999**, *321*, 1-3; (b) Babu, B. S.; Balasubramanian, K. K. *Tetrahedron Lett.* **2000**, *41*, 1271-1274; (c) Mukherjee, D.; Yousuf, S. K.; Taneja, S. C. *Tetrahedron Lett.* **2008**, *49*, 4944-4948; (d) Ghosh, R.; Chakraborty, A.; Maiti, S. *Arkivoc* **2004**, *xiv*, 1-9.
5. (a) Lefever, M. R.; Szabò, L. Z.; Anglin, B.; Ferracane, M.; Hogan, J.; Cooney, L.; Polt, R. *Carbohydr. Res.* **2012**, *351*, 121-125; (b) Ghosh, R.; Chakraborty, A.; Maiti, D. K. *Synth. Commun.* **2003**, *33*, 1623-1632.
6. (a) Ravindranathan Kartha, K. P.; Field, R. A. *Tetrahedron Lett* **1997**, *38*, 8233-8236; (b) Ercegovic, T.; Meijer, A.; Magnusson, G.; Ellervick, U. *Org. Lett.* **2001**, *3*, 913-915.
7. (a) Meijer, A.; Ellervick, U. *J. Org. Chem.* **2002**, *67*, 7407-7412; (b) Cura, P.; Aloui, M.; Ravindranathan Kartha, K. P.; Field, R. A. *Synlett* **2000**, *9*, 1279-1280.
8. Meijer, A. Development of a Novel Promoter System for Thioglycoside Activation. Ph.D. Dissertation [Online], Lund University, Lund, Sweden, December 2003. http://www.organic.lu.se/people/ulf%20ellervik/ellervikresearchgroup/Meijer.pdf (accessed Dec 20, 2013)

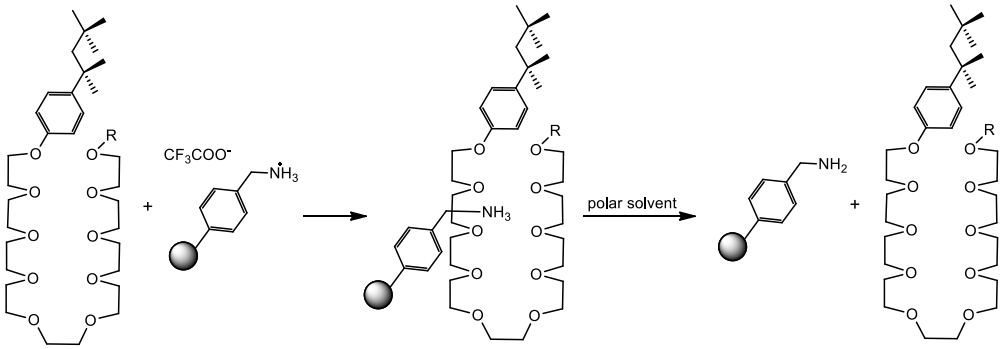
Chapter III Synthesis of Sia α-(2→6) Gal using Affinity Separation

Introduction

In normal solution phase sialylation reactions, separation of the product from excess sialyl donors and its derivatives is usually difficult because of the similarities in their polarities on silica-gel. Thus, other purification techniques such as high-performance liquid chromatography (HPLC) are usually employed. Purification by HPLC is, however, limited by its small scale. To address this, we turned to phase tagging as an alternative separation method.

In phase tagging, the reaction between the tag and target is done in solution while the subsequent purification step utilizes a resin as in solid phase synthesis. Tags for this strategy range from PEG compounds to fluorous alcohols and other hydrophobic substances. In a previous investigation in our group, 32-crown-10 ethers, and podand tags with polyethylene glycol (PEG) moieties functionalized with aminobenzyl-type linkers were used as tags while polystyrene resins with aminomethyl groups, such as ArgoPore-NH_2, were used as the column stationary phase for the separation.[1] Using this separation technique, several oligosaccharides have been synthesized. However, since the crown ether, and podand-type tags still have to be synthesized prior to attachment with the substrate, we tried to look for other tags that can readily be used with little or no modification needed prior to attachment. We then turned our attention to the plausible use of Triton X-100 as a tag because of its PEG component.

Triton X-100 is a mild, non-denaturing, nonionic surfactant with a clear to slightly yellowish tint. It is an octylphenol ethoxylate with an average of 9.5 ethylene oxide units per molecule. Since it is heterogeneous, higher and lower molecular weight polymers (ranges from 6 – 15 ethylene oxide units) are present in smaller amounts. This surfactant is also miscible in water, toluene, xylene, trichloroethylene, ethylene glycol, ethyl ether, ethanol, isopropanol, ethylene dichloride, tetrahydrofuran but immiscible in kerosene and mineral spirits.[2] It absorbs in the ultraviolet region of the spectrum and most of existing literature makes use of Triton as a component of cell lysis buffers or other solutions for the extraction and solubilization of proteins. For this purpose, a 0.1% solution in water is usually enough although many enzymes, such as Proteinase K, remain active even in higher concentrations.[3]

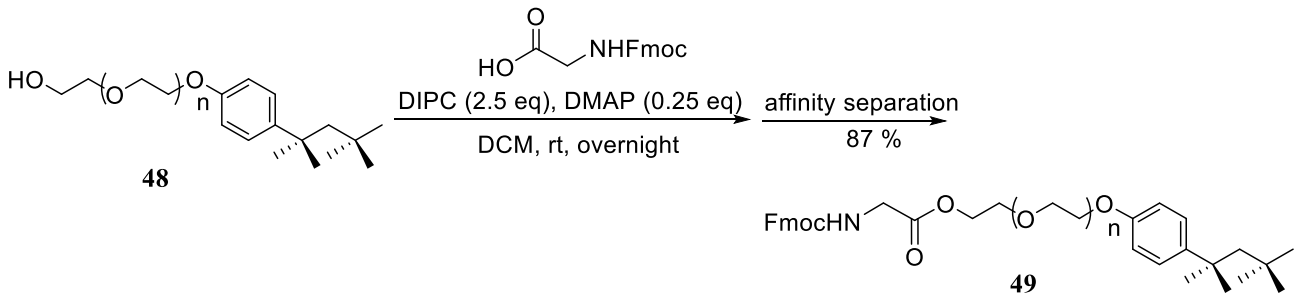


Scheme III-1. Synthesis based on Affinity Separation using Triton X-100 and ArgoPore-NH_2.

Nonpolar solvents such as CH_2Cl_2 can be used to allow Triton adsorption on ArgoPore-NH_2 columns previously treated with trifluoroacetic acid (TFA), as untagged substances are washed away. This is followed by desorption of the tagged compound using polar solvents such as CH_2Cl_2-CH_3OH (Scheme III-1).[4]

Affinity Separation Model Study

As a preliminary investigation in the use of affinity tags, Triton X-100 was coupled with Fmoc-protected glycine using DIPC followed by affinity separation (Scheme III-2). The tagged product was obtained after washing with CH_2Cl_2-CH_3OH (1:1) in 87% yield with excellent purity.

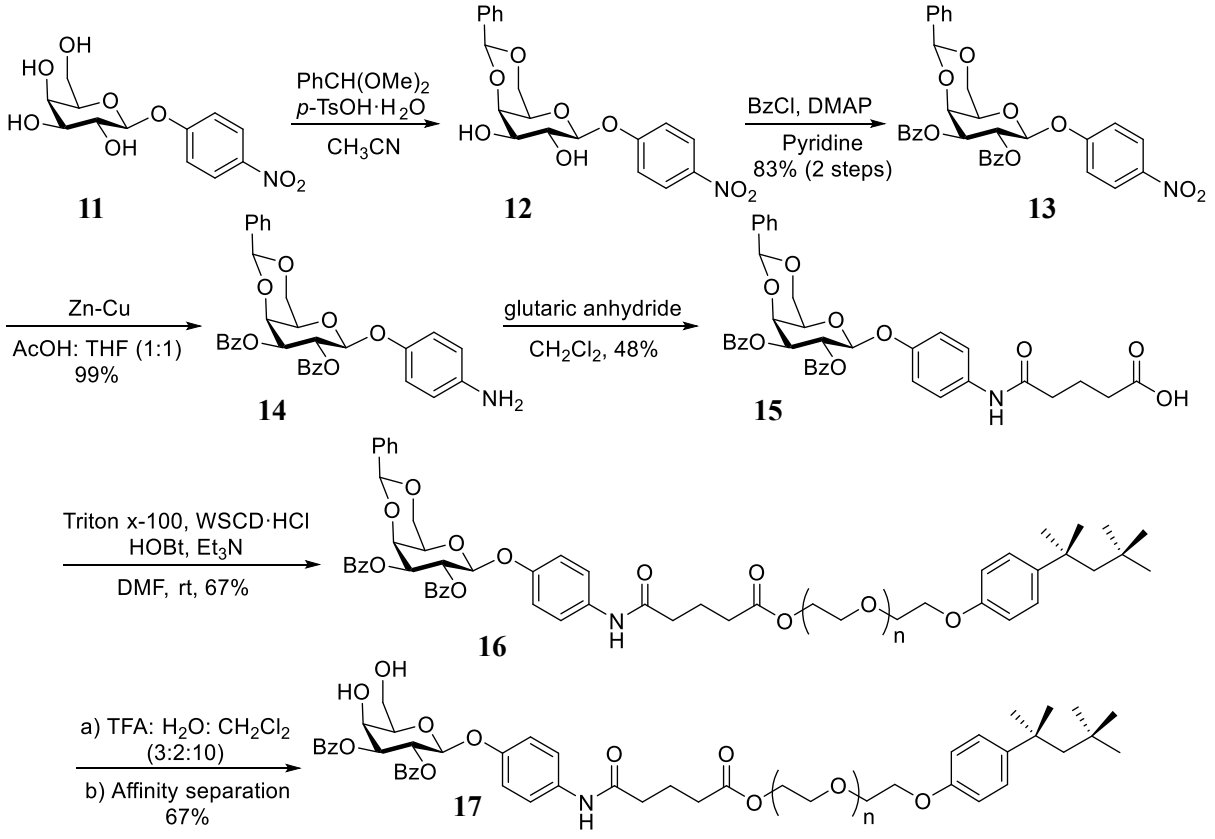


Scheme III-2. Model study using Triton X-100 as affinity tag.

Because of this result, the use of Triton as an affinity tag in sialic acid synthesis was investigated. If the conditions for In(III)-mediated sialylation can be applied using the phase tagging system, the difficulty in the separation of decomposed sialic acid donor, which has a similar polarity with the disaccharide bearing sialic acid, will be circumvented.

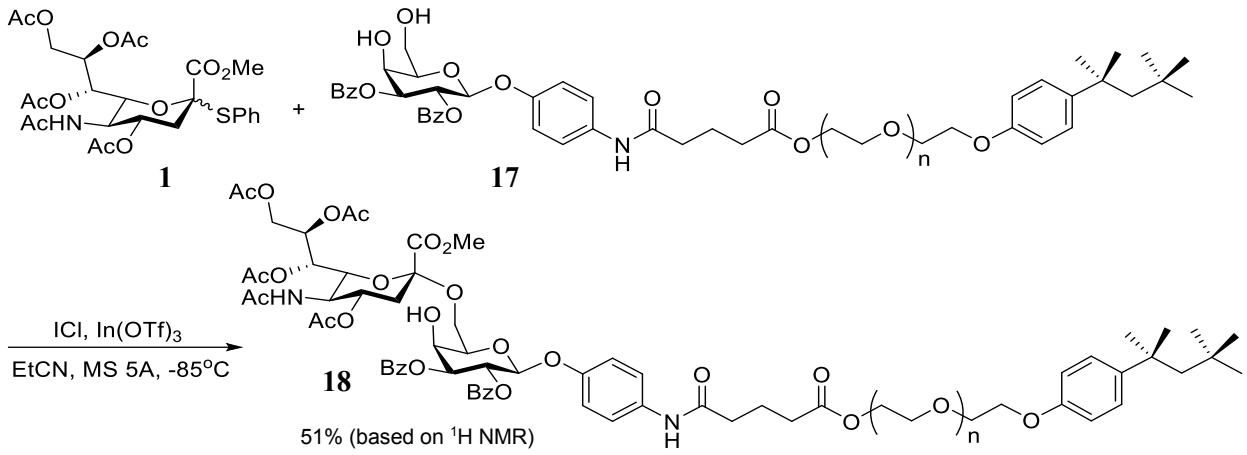
Synthesis of Triton-linked Monosaccharide

Commercially available *p*-nitrophenyl-galactopyranoside was protected with benzaldehyde dimethyl acetal at the 4- and 6-positions followed by benzoylation at the 2- and 3-positions (Scheme III-3). The nitro substituent of the aromatic ring was then reduced to an amine using zinc-copper couple in acetic acid/tetrahydrofuran. The resulting compound was reacted with glutaric anhydride in DCM.[6] Subsequently, condensation with Triton tag was done using WSCD·HCl, HOBt, and triethylamine to give the tagged monosaccharide in 67% yield. This was purified by silica gel column chromatography instead of ArgoPore-NH_2 because attachment of glutaric anhydride rendered the sugar insoluble in DCM. Thus, even if the ArgoPore-NH_2 column is washed with DCM, the excess starting material will not be eluted and will still come out together with the tagged product after desorption with DCM/MeOH. It should also be noted that after the Triton tag was attached to the sugar, it became soluble in DCM again allowing the deprotection of the benzylidene protecting group using trifluoroacetic acid: water: dichloromethane. In this case, purification was done using ArgoPore-NH_2 columns to give the 4,6-free hydroxyl galactoside acceptor in 67% yield.



Scheme III-3. Synthesis of Triton-linked galactoside acceptor.

α(2→6) Sialylation using IC/In(OTf)$_3$



Scheme III-4. Synthesis of Triton-tagged Sia α(2→6) Gal.

The conditions for α-sialylation using ICl/In(OTf)$_3$ promoter system discussed in the previous section was applied in activating the thioglycoside donor. The starting materials were only dried in vacuo prior to the reaction without lyophilization. Purification was done using ArgoPore-NH_2 columns with the Triton-linked disaccharide eluting from the column after washing with DCM/MeOH (1:1) in 51% yield (Scheme III-4). After affinity separation, the disaccharide was readily separated from sialyl donor derivatives, and excess acceptor. Further functionalization of the disaccharide can be done by cleavage of the *para*-acylaminophenyl glycosidic linkage via ceric ammonium nitrate (CAN) oxidation.[1b]

Summary

In this chapter, the application of the previously investigated promoter system in the synthesis of a tagged disaccharide was discussed. Triton x-100 was used as the affinity tag due to its interaction with $–NH_3^+$ on ArgoPore beads that were pre-washed with TFA. After the sample addition, excess reagents were washed with DCM while the Triton-tagged sugar was eluted with DCM/MeOH. Attachment of Triton also helped improve the solubility of the sugar which is advantageous in solution-phase synthesis. In the end, purification using column chromatography was almost not needed after tag attachment which reduced the amount of time needed for the synthetic work.

References:

1. (a) Zhang, S.-Q.; Fukase, K.; Kusumoto, S. *Tetrahedron Lett*. **1999**, *40*, 7479-7483; (b) Fukase, K.; Takashina, M.; Hori, Y.; Tanaka, D.; Tanaka, K.; Kusumoto, S. *Synlett*. **2005**, *15*, 2342-2346. (c) Fukase, K.; Zhang, S.-Q.; Fukase, Y.; Umesako, N.; Kusumoto, S. In *New Discoveries in Agrochemicals*; Clark, J. M.; Ohkawa, H., Eds; ACS Symposium Series 892, American Chemical Society: Washington DC, **2005**, 87-98.
2. (a) Triton x-100™. *Sigma Product Information Sheet*; http://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Product_Information_Sheet/1/t8532pis.pdf (accessed May 28, 2013); (b) Triton non-ionic surfactant x-100. *Shun Chia Industrial Company Limited Product Description* (accessed May 28, 2013); (c) Thermo Scientific: Pierce Protein Biology Products. *Triton x-100 Detergent Solution*; http://www.piercenet.com/product/ triton-x-100-detergent-solution (accessed May 28, 2013).
3. Johnson, M. Detergents: Triton X-100, Tween-20, and More. *Labome: Material and Methods*; http://www.labome.com/method/Detergents-Triton-X-100-Tween-20-and-More.html (accessed May 28, 2013).
4. Kaufmann, G.; Sartorio, R.; Lee, S-H.; Mee, J. M.; Altobell, L. J., III; Kujawa, D. P.; Jeffries, E.; Clapham, B.; Meijler, M. M.; Janda, K. D. *J. Am. Chem. Soc.* **2006**, *128*, 2802-2803.

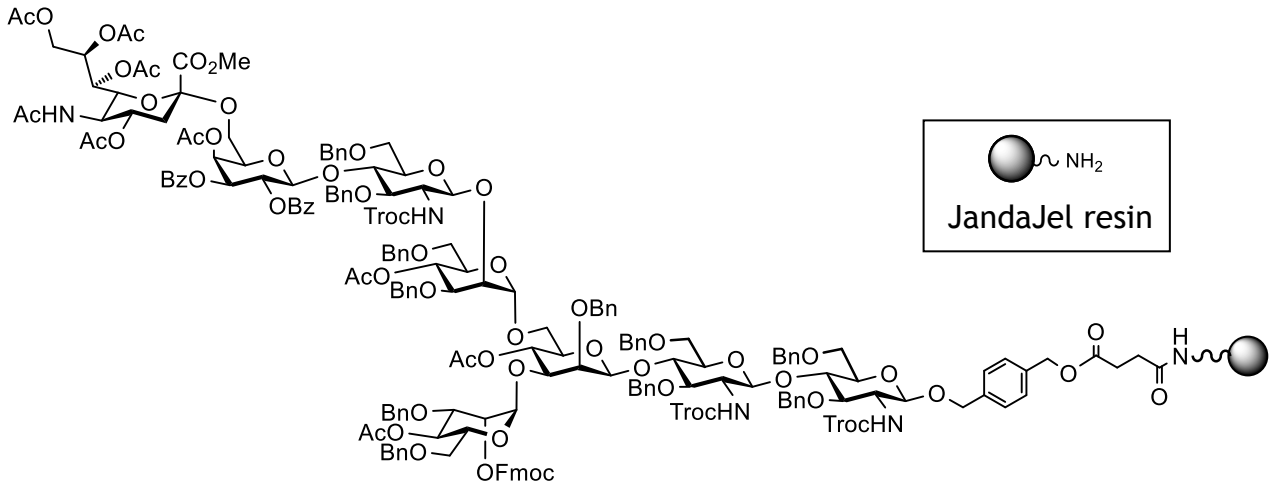
Chapter IV Development of Linkers for Solid Phase Synthesis

Introduction

Aside from the choice of resin, the type of linker to be used for solid-phase synthesis also plays a crucial role in this kind of strategy. The kind of functional groups in the linker not only dictate the ease of its attachment to the oligosaccharide, but the method of its cleavage from the resin as well. Over the years, several groups have introduced their own linkers as well as cleavage method. Ogawa, for one, attached *p*-allyloxybenzyl alcohol to a disaccharide and used it as a linker for the loading of the disaccharide onto Merrifield resin.[1] After elongation of the glycan chain, the oligosaccharide was cleaved from the resin using $TrBF_4$ in DCM.

Nicolau's group, on the other hand, used a nitrobenzyl ether-type linker by reacting 5-hydroxy-2-nitrobenzaldehyde with 1,3-diiodopropane followed by reduction with $NaBH_4$. After glycosylation of the resulting iodobenzyl alcohol linker with the monosaccharide, the product was loaded onto a polystyrene resin. In this case, cleavage was done by irradiation of the oligosaccharide-containing resin at 25°C in THF.[2] While a recent report by Seeberger discussed the use of an acylsulfonamide safety-catch linker attached onto TentaGel resin for the synthesis of sialyl LewisX tetrasaccharide.[3]

In our group, *p*-xylylene glycol has been used as a linker in the solid-phase synthesis of a pentasaccharide and sialic acid-containing octasaccharide (Scheme IV-1).[4]



Scheme IV-1. Solid-phase synthesis of sialic acid-containing octasaccharide.

In the current work, several acid-stable linkers for solid-phase oligosaccharide synthesis were investigated. The linker was attached onto JandaJel resin followed by the loading of asparagine. *N*-glycosylation using *N*-

(phenyl) trichloroacetimidates was carried out using TMSOTf as activator in order to attach the first building block (Figure IV-1).

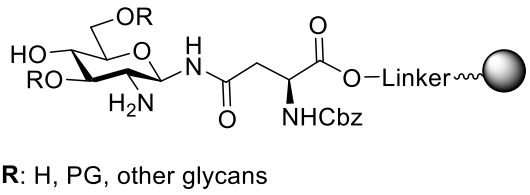
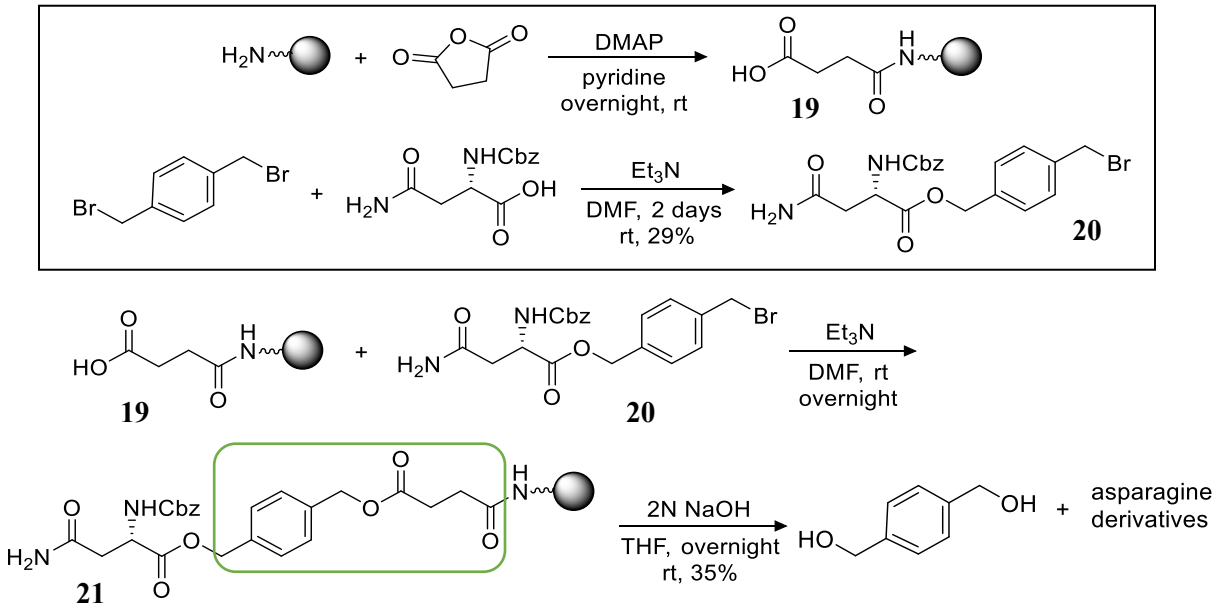


Figure IV-1. Target structure for the development of solid-phase linkers.

Synthesis of Benzyl ester Linker

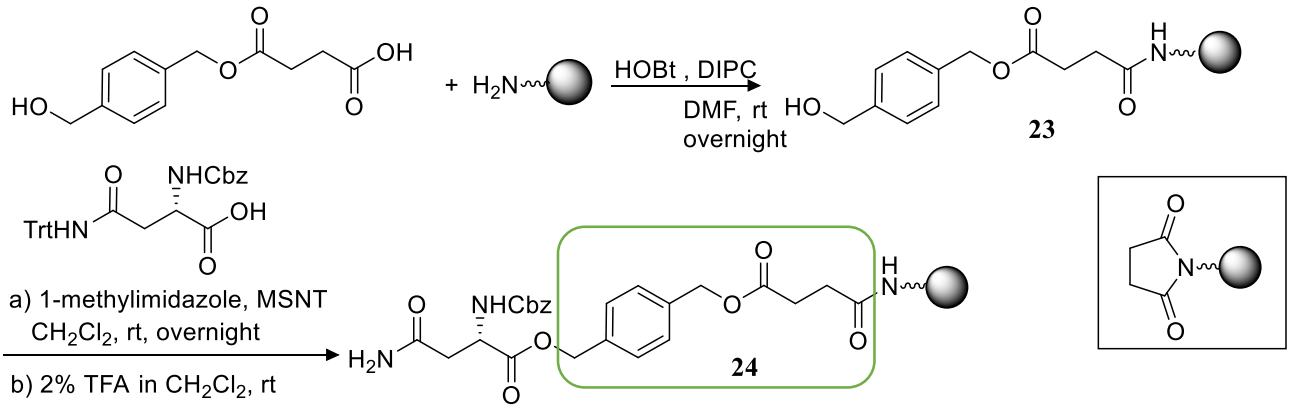
Several kinds of benzyl ester were developed, and their cleavage conditions investigated. In the first route, JandaJel-NH_2 resin was further functionalized by reacting with succinic anhydride in the presence of DMAP while asparagine was reacted with 1,4-bis-bromomethylbenzene (Scheme IV-2). The products of the two separate reactions were then reacted in the presence of triethylamine to attach asparagine onto the solid support via the benzyl ester linker. When the asparagine was released from the resin using 2 N NaOH to give *p*-xylylene glycol in 35% yield. Even though the synthesis was simple enough, the main drawback of this synthetic route was that, the esterification reaction was not very efficient.



Scheme IV-2. Benzyl ester linker synthesis using succinic anhydride.

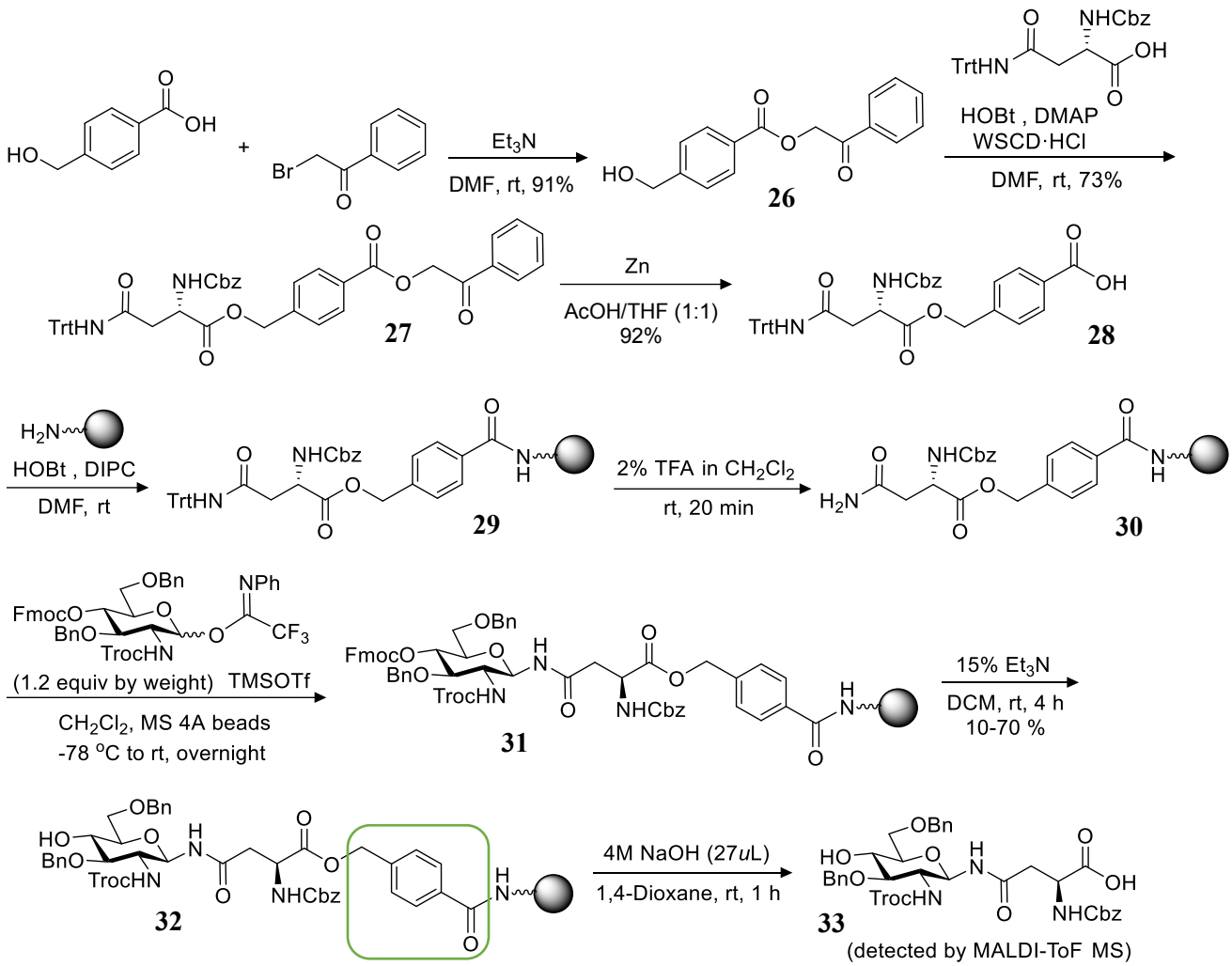
In an attempt to circumvent the inefficient esterification step, a second synthetic route was carried out where the benzyl ester linker was directly loaded onto JandaJel-NH_2 resin using DIPC and HOBt (Scheme IV-3). After washing with alternately with DCM and MeOH, trityl-protected asparagine was attached using 1-methylimidazole and MSNT. Then the trityl group was deprotected using TFA. However, the conditions during

the coupling reactions could have also facilitated the attack of the nitrogen atom in JandaJel resin on the ester moiety of the linker which can result in cyclization (inset Scheme IV-3).



Scheme IV-3. Direct loading of benzyl ester linker onto JandaJel resin.

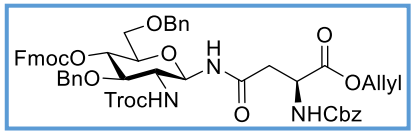
Another synthetic route to synthesize a slightly shorter benzyl ester linker starting from the condensation of 4-(hydroxymethyl)benzoic acid and 2-bromoacetophenone was undertaken (Scheme IV-4). This was followed by coupling with trityl-protected asparagine then deprotection of the phenacyl ester using zinc in acetic acid/tetrahydrofuran to give the deprotected product. This was loaded onto JandaJel beads using DIPC and HOBt. After washing with DCM and MeOH, the trityl group was deprotected using 2% TFA in DCM. The resin was washed thoroughly with DCM and MeOH prior to drying in vacuo. *N*-glycosylation was done using *N*-(phenyl)trifluoroacetimidate glycosyl donor activated by TMSOTf at low temperature overnight. The extent of monosaccharide loading was determined by cleaving the Fmoc protecting group using 15% Et_3N followed by UV analysis of the resulting 9-methylene-9*H*-fluorene chromophore. This was found to give inconsistent results, thus, the glycosyl asparagine moiety had to be cleaved from the resin.



Scheme IV-4. Linker synthesis using 4-(hydroxymethyl)benzoic acid and 2-bromoacetophenone.

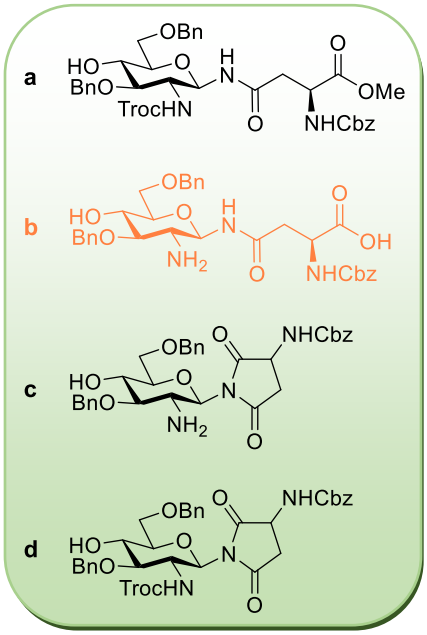
An investigation of cleavage condition using various solvents that can swell JandaJel beads was done (Table IV-1). The investigation, which was done in solution phase, involved the deprotection of the ally-group from glycosyl asparagine by treating it with varying concentrations of NaOH in different solvents. From entries 1-3, it can be seen that the desired product was obtained in combination with cyclic imides when 1 M NaOH was used with either THF or 1,4-dioxane as solvent. Because JandaJel-NH_2 resin swells more in 1,4-dioxane, this was used as the solvent while the concentration of NaOH was varied. Entries 4 and 5 gave a similar result as that of entry 3. Entry 6, on the other hand, furnished on the desired allyl-deprotected product. Thus, this condition was employed in the cleavage of glycosyl asparagine from the resin.

Table IV-1. Investigation of cleavage condition.



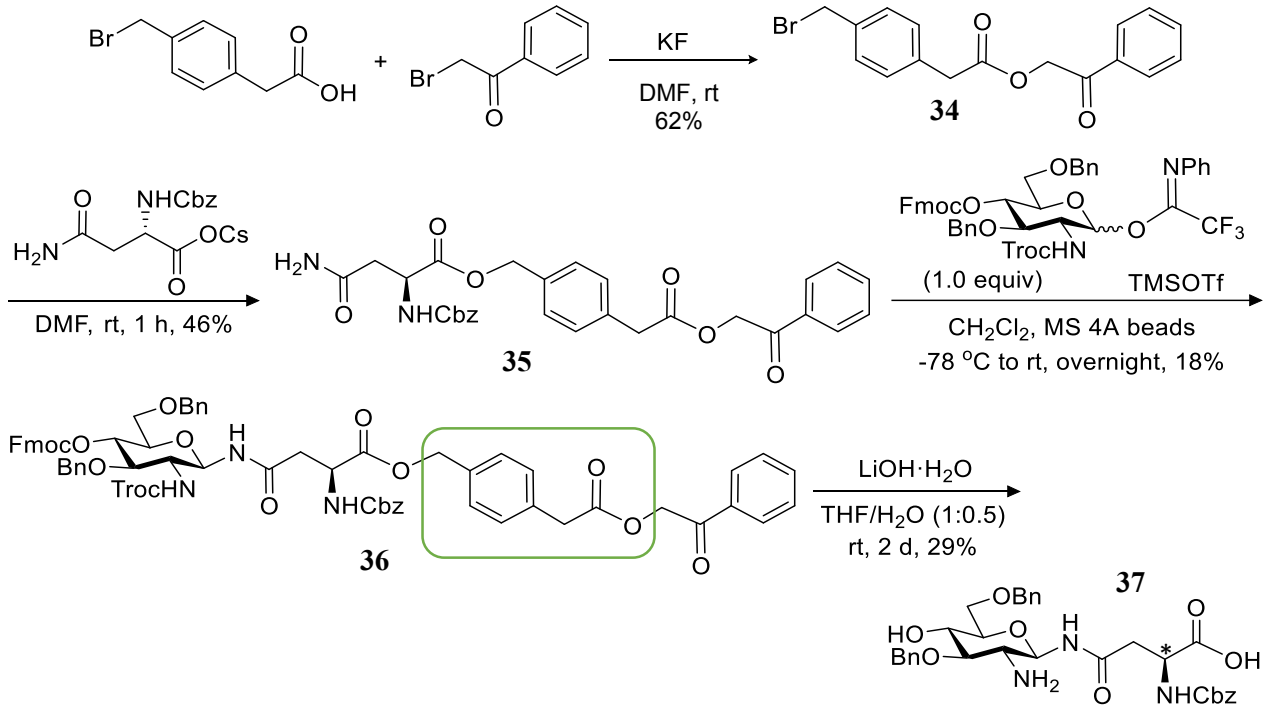
Entry	Base (27µL)	Solvent (1.92 mL)	Time (h)	Product[a]
1	1M NaOH	MeOH	1-3	a
2	1M NaOH	THF	1-3	b, c
3	1M NaOH	1,4-Dioxane	1-3	b, d
4	2M NaOH	1,4-Dioxane	1	b, d
5	3M NaOH	1,4-Dioxane	1	b, d
6	4M NaOH	1,4-Dioxane	1	b

a: based on MALDI-ToF MS data; Compound b: desired product.



However, the product was only obtained in trace amount. Although glycosylation did proceed on the solid support, the very small amount of product obtained considering the number of steps to be undertaken as well as the possible isomerization of asparagine by using NaOH, prompted not only the search for a new synthetic route but a re-investigation of the cleavage reaction as well.

For the fourth synthetic route, 4-(bromomethyl)phenylacetic acid was reacted with 2-bromoacetophenone followed by the addition of the cesium salt of asparagine (Scheme IV-5). Afterwards, *N*-glycosylation with *N*-(phenyl) trifluoroacetimidate glycosyl donor was done using TMSOTf as an activator to give the linker-bound monosaccharide in 18% yield. A reinvestigation of the cleavage conditions from the resin using *N*-carbobenzyloxy-β-trityl-L-asparagine benzyl ester as substrate, and TBAOH in THF, TEAH in THF, 4 M NaOH in 1,4-dioxane, and LiOH·H_2O in THF/H_2O as cleaving agents was done. Of these, LiOH·H_2O in THF/H_2O gave the desired benzyl ester-deprotected product, and was thus used in the deprotection of compound **36** which resulted in 29% yield, however isomerization was still observed with the desired product to isomer ratio of 4:1.



Scheme IV-5. Benzyl ester synthesis using Br compounds.

Synthesis of Allyl Linker

With the use of the benzyl ester linker, it was observed that glycosylation indeed proceeded on the solid support, however the cleavage from the resin became a persistent problem. Obtaining the product in trace amount after cleavage as well as the possible isomerization of asparagine prompted the search for a new linker strategy which is cleavable without the use of a strong base. The use of allyl groups (Figure IV-2) was thought of as an interesting alternative as it can be constructed in several ways and cleaved using palladium catalysts.

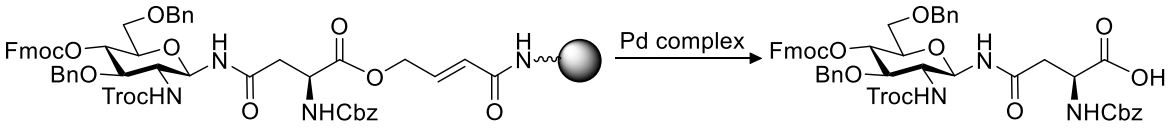
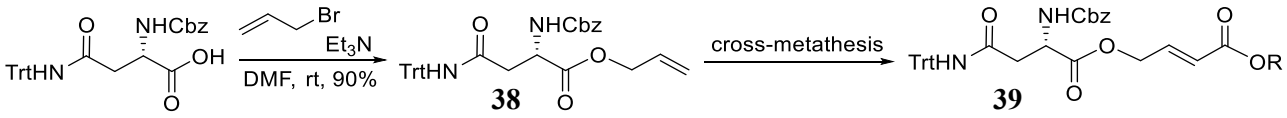


Figure IV-2. Allyl linker strategy.

In the first route, allyl bromide was reacted with asparagine to give the product in 90% yield followed by cross-metathesis reaction with acrylic acid or methyl acrylate using second generation Grubbs catalyst (Table IV-2). Entries 1-3 show that using acrylic acid, copper iodide and Grubbs-2 catalyst in much excess, or prolonging the reaction time facilitated the formation of only a trace amount of product. Changing the solvent

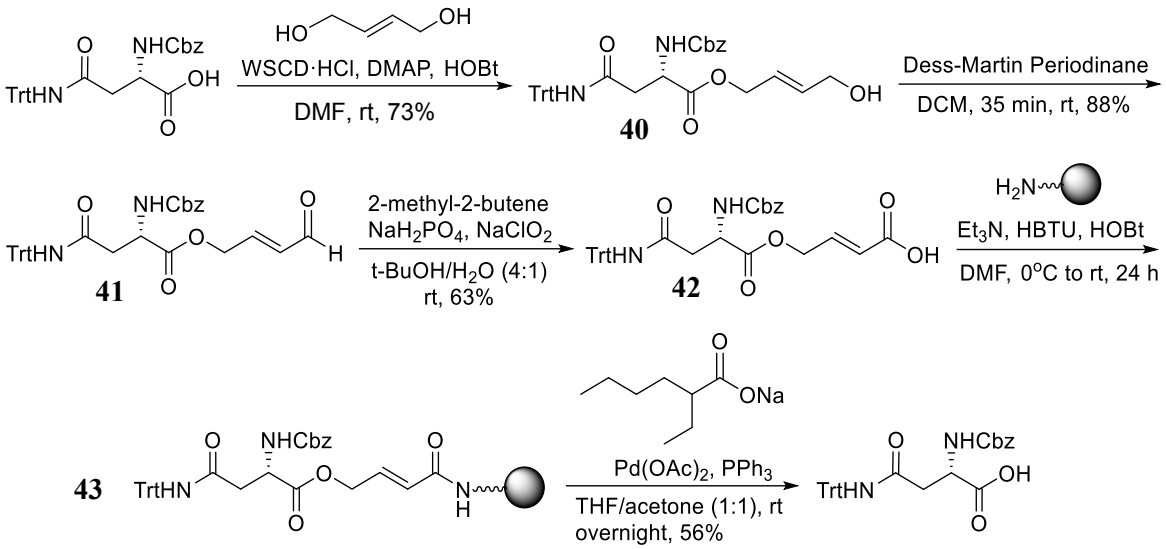
to toluene, on the other hand, favored homo-coupling (entries 4-5). Only when methyl acrylate was used as the solvent did a considerable amount of product form. To improve the yield, the amount of catalyst was increased. Unfortunately, this only resulted in a much lower yield. Because of low yields and the high cost of metal catalysts, an easier and cheaper way to synthesize the allyl linker was sought.

Table IV-2. Allyl linker synthesis via cross-metathesis reaction.



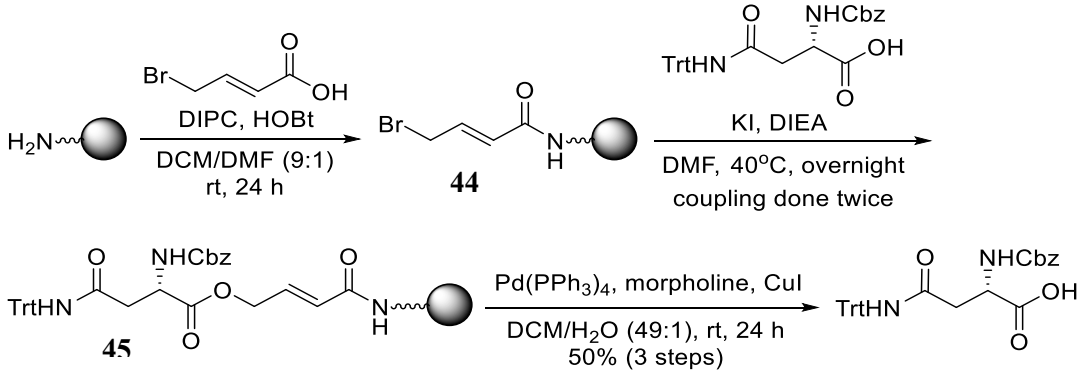
Entry	*R*	*Reagents*	*Solvent*	*Temperature time*	*Reaction*	*Results*
1	H	acrylic acid (3 eq), CuI (3mol%), Grubbs-2 cat. (2mol%)	$C_2H_5OC_2H_5$	40°C reflux	3h	no reaction
2	H	acrylic acid (6 eq), CuI (3mol%), Grubbs-2 cat. (4mol%)	CH_2Cl_2	40°C reflux	19h	trace
3	H	acrylic acid (6 eq), CuI (6mol%), Grubbs-2 cat. (8mol%)	$C_2H_4Cl_2$	90°C reflux	6h	trace
4	H	acrylic acid (6 eq), CuI (6mol%), Grubbs-2 cat. (8mol%)	Toluene	120°C reflux	2h	trace homo-coupling
5	CH_3	methyl acrylate (3 eq), CuI (3mol%), Grubbs-2 cat. (2mol%)	Toluene	120°C reflux	2h	trace homo-coupling
6	CH_3	Grubbs-2 cat. (12mol%)	Methyl acrylate	90°C reflux	6.5h	27%
7	CH_3	Grubbs-2 cat. (30mol%)	Methyl acrylate	90°C reflux	3h	19%

For the second route, condensation of trityl-protected asparagine with 2-butene-1,4-diol was done using WSCD·HCl, DMAP and HOBt to give the product in 76% yield (Scheme IV-6). The alcohol was then oxidized to an aldehyde using DMP followed by further conversion of the aldehyde to a carboxylic acid using Pinnick oxidation. Attachment of the product to JandaJel beads via amide formation was done using triethylamine, HBTU and HOBt at lower temperature. To determine the amount of asparagine loaded onto the resin, sodium 2-ethylhexanoate, palladium (II) acetate and triphenylphosphine[5] were used in the cleavage reaction which gave trityl-protected asparagine in 56% yield.



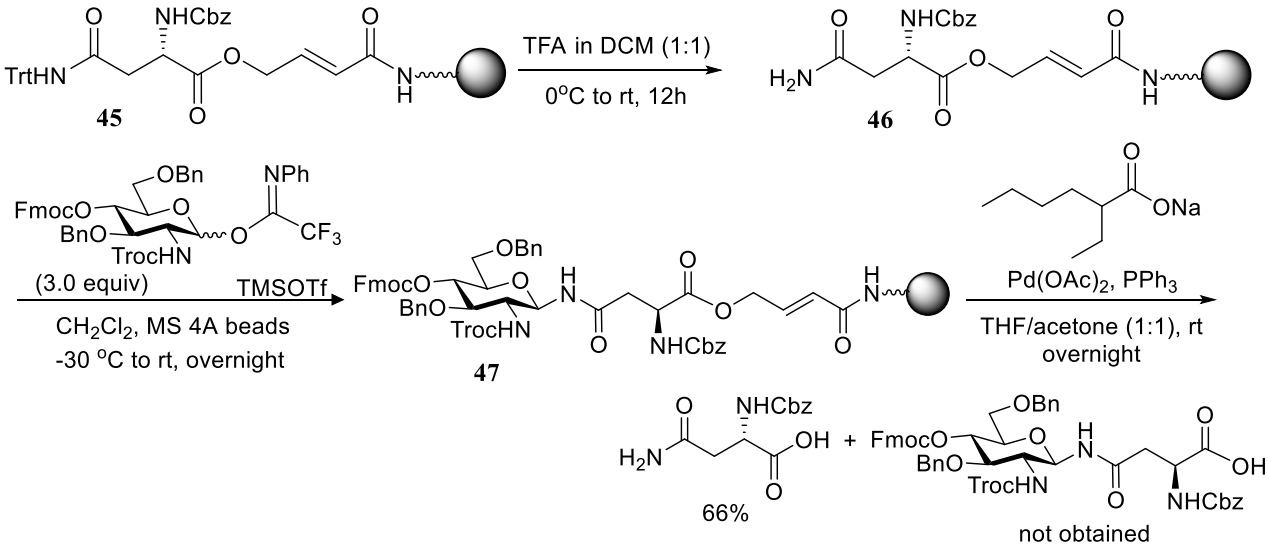
Scheme IV-6. Allyl linker synthesis via allylic alcohol oxidation.

To achieve a more efficient synthesis, the third route involving the further functionalization of JandaJel beads by condensation with 4-bromocrotonic acid using DIPC and HOBt in mixed DCM/DMF solvent (Scheme IV-7) was investigated. After shaking for 24 hours, the resin was washed and dried in vacuo and then utilized for the next reaction where trityl-protected asparagine was attached using KI and DIEA at elevated temperature. Iteration was done to ensure effective loading. Cleavage from the resin was done using tetrakis(triphenylphosphine)palladium (0), morpholine and copper iodide[6] to give the trityl-protected asparagine in 50% yield. This yield is comparable with that of the second route but with fewer steps and direct resin introduction of the substrates, thus eliminating the need for column chromatography of the intermediates.



Scheme IV-7. Ally linker synthesis via 4-bromocrotonic acid.

Because of this, *N*-glycosylation was then done on solid support using the allyl linker (Scheme IV-8). The trityl group was deprotected using TFA in dichloromethane followed by *N*-glycosylation, then cleavage from the JandaJel resin using palladium (II) acetate. However, only the trityl-deprotected asparagine was obtained.



Scheme IV-8. *N*-glycosylation on solid support using allyl linker.

Even though glycosylation reproducibility on solid support was low, these linkers are not restricted to solid-phase synthesis. Coupling these linkers with the phase tagging system discussed in Chapter III lends an alternative strategy for *N*-glycan synthesis. This way, the inherent disadvantages of solid-phase synthesis in real-time monitoring, and imposition of strict condition control can be circumvented, while still allowing the easy separation of the product.

Summary

Several synthetic routes in the preparation of some benzyl ester and allyl-type linker were discussed. Relatively short steps were required in the synthesis of most of the linkers taking into account commercial availability, ease of handling and cost. In general, two strategies were employed in linker introduction: condensation of the linker with asparagine prior to resin loading, or direct attachment of the linker onto the resin before the reaction with asparagine. The propensity of asparagine to isomerize became one of the main challenges of this work since cleavage was usually done using a base. Strict temperature control was also quite difficult when *N*-glycosylation reactions were performed due to the heterogeneity of the reaction mixture. Nevertheless, the linkers discussed in this section were intended to, but are not exclusive, for solid phase synthesis, thus, their application in triton tagging system can also be explored.

References:

1. Shimizu, H.; Ito, Y.; Kanie, O.; Ogawa, T. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2841-2846.
2. Nicolau, K. C.; Winssinger, N.; Pastor, J.; DeRoose, F. *J. Am. Chem. Soc.* **1997**, *119*, 449-450.
3. Yin, J.; Eller, S.; Collot, M.; Seeberger, P. H. *Beilstein J. Org. Chem.* **2012**, *8*, 2067-2071.
4. Tanaka, K.; Fujii, Y.; Tokimoto, H.; Mori, Y.; Tanaka, S.; Bao, G-m.; Siwu, E. R. O.; Nakayabu, A.; Fukase, K. *Chem. Asian J.* **2009**, *4*, 574-580.
5. Jeffrey, P. D.; McCombie, S. W. *J. Org. Chem* **1982**, *47*, 587-590.
6. Tulla-Puche, J.; Barany, G. *Tetrahedron* **2005**, *61*, 2195-2201.

Chapter V Conclusion

In this study, α(2→6) sialylation was achieved using ICl/In(OTf)$_3$ as promoter system in activating the thiophenyl sialic acid donor with the *O*-propyl galactoside acceptor (Figure 1). Even though the reaction proceeded in the presence of moisture, conducting the reaction under inert atmosphere in the presence of molecular sieves improved the yield. This promoter system was also found to work well with highly reactive acceptors.

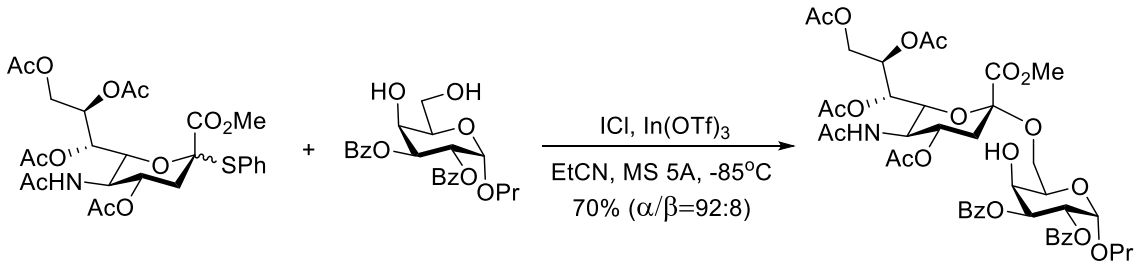
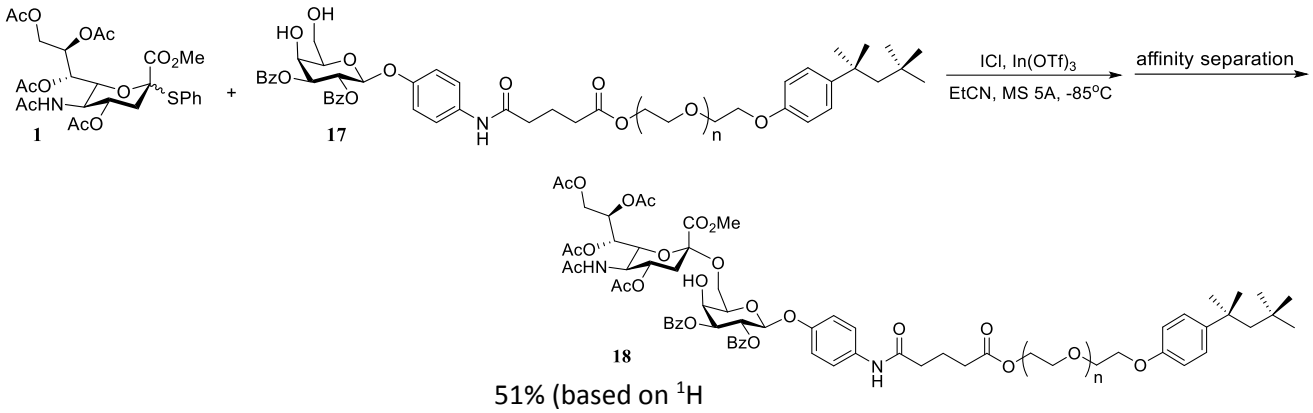


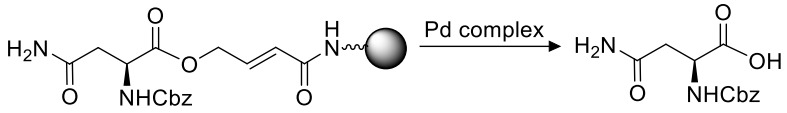
Figure 1. ICl/In(OTf)$_3$-promoted α(2→6) sialylation.

α-Sialylation using ICl/In(OTf)$_3$ was also applied in phase tag strategy. Triton x-100 was used as an affinity tag and attached to the protected galactoside acceptor. Attachment of triton improved the solubility of the acceptor allowing it to be dissolved again in the organic solvents utilized in the reaction and purification steps. The triton-tagged sialic acid-containing disaccharide was purified using ArgoPore-NH_2 columns which were pre-washed with TFA in DCM. The molecular interactions of the $–NH_3^+$ groups on the column with the PEG component of the triton tag (Scheme 1) allowed the easy separation of the product by desorption using DCM/MeOH after the excess reagents have been washed using DCM.



Scheme 1. Affinity separation using Triton x-100 and ArgoPore-NH_2.

Linkers for solid phase synthesis were also developed. Several benzyl ester, and allyl linkers were prepared either by attaching them to asparagine or directly loading onto JandaJel resin.



Scheme 2. Allyl linker.

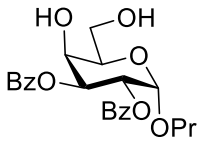
Asparagine was found to isomerize during the cleavage reaction when the benzyl ester-type linkers were employed so allyl linkers presented viable solutions (Scheme 2). The allyl linker can instead be cleaved using palladium compounds. The utility of these linkers albeit developed initially for the use of solid supports, are not restricted to solid phase synthesis. Since these linkers are soluble in most organic solvents, their application in orthogonal solution phase synthesis is something that can also be investigated in the future.

Chapter VI Experimental Procedures

General

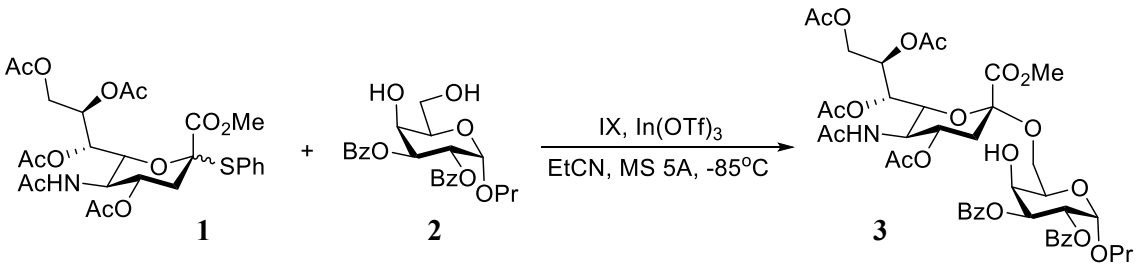
^{1}H NMR spectra were recorded in indicate solvents using JEOL ECA-500 spectrometers. The chemical shifts in $CDCl_3$ are given in *d* values from tetramethylsilane (TMS) as an internal standard. High resolution mass spectrometry was performed using LTQ-Orbitrap XL. Solid-phase synthesis shaker used was Peti-Syzer PSS-510 (HiPep Laboratories) in combination with EYELA NCB-1200 (Tokyo Rikakikai Co. Ltd.) temperature controller. Silica-gel column chromatography was done using Kieselgel 60 (Merck, 0.040-0.063 mm), or Silica Gel 60 N (Kanto Chemical Co., spherical, neutral 0.040-0.050 mm or 100-210 μm) at medium pressure (2-4 kg/cm^2). Precoated Kieselgel 60 F_{254} (Merck Co., 1 mm, or 0.5 mm) was used for preparative thin-layer chromatography. TLC was performed on Silica-gel F_{254} (Merck), compound visualization using UV (254 nm), and staining using phosphomolybdic acid solution (5% in EtOH), 0.03% *p*-methoxybenzaldehyde in EtOH-H_2SO_4 (conc.)-AcOH, or 0.2% ninhydrin in EtOH-collidine-acetic acid. Molecular sieves 4Å were activated by heating at 250°C in vacuo for 3 h prior to use, while molecular sieves 5Å were activated by microwave irradiation at 440 W for 1 min, then drying in vacuo (3 times). Non-aqueous reaction were performed under argon atmosphere unless noted otherwise. Anhydrous CH_2Cl_2 was distilled from calcium hydride. Anhydrous THF was purchased from Kanto Chemicals, Tokyo, Japan, while anhydrous DMF was purchased from Nacalai Tesque Inc., Kyoto, Japan. Distilled water was prepared by a combination of Arium® 611 UV (Sautorius) or Toray Pure LV-308 (Toray) and GSL-200 (Advantec, Tokyo, Japan). All other reagents and solvents were also purchased from commercial sources.

Experiments in Chapter II



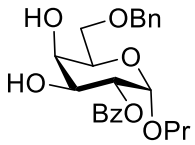
Propyl 2,3-di-*O*-benzoyl-α-D-galactopyranoside (2)

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01-7.98 (m, 4 H), 7.54-7.49 (m, 2 H), 7.40-7.36 (m, 4 H), 5.73-5.67 (m, 2 H), 5.31 (d, J = 3.5, 1 H), 4.47 (m, 1 H), 4.10 (t, J = 4.5, 1 H), 4.04-4.00 (m, 1 H), 3.96-3.91 (m, 1 H), 3.74-3.70 (m, 1 H), 3.43-3.39 (m, 1 H), 2.86 (s, 1 H), 2.24 (s, 1 H), 1.64-1.58 (m, 2 H), 0.90 (t, J = 7.5, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.1, 165.9, 133.4, 133.2, 129.8, 129.5, 129.4, 128.5, 128.4, 96.6, 71.2, 70.2, 69.9, 69.0, 68.9, 63.4, 22.7, 10.6; HRMS (LTQ-Orbitrap XL, positive) Anal. Calculated for $C_{23}H_{26}O_8Na_1$ $[M+Na]^+$: 453.1520, found 453.1526.



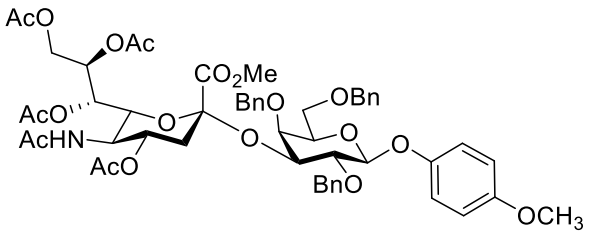
Representative procedure for IX/In(OTf)$_3$-promoted sialylation (3).

To a solution of donor **1** (101.7 mg, 0.174 mmol), and acceptor **2** (50 mg, 0.116 mmol) in EtCN (2.26 mL) was added ICl (54.7 mg, 0.337 mmol) at -85°C followed by the addition of In(OTf)$_3$ (131 mg, 0.232 mmol). The reaction was stirred for 3 h and monitored by TLC. The reaction was quenched with Et_3N (0.68 mL), then warmed to room temperature followed by filtration Hyflo Super-Cel® and concentration in vacuo. Silica-gel column chromatography (1 g, toluene:EtOAc = 1:2) to get **3** (74 mg, 70%, BRSM: 85%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01 (dd, J = 19.5, 7.2 Hz, 4 H), 7.52-7.48 (m, 2 H), 7.39-7.35 (m, 4 H), 5.67-5.66 (m, 2 H), 5.39-5.33 (m, 2 H), 5.28 (s, 1 H), 5.23-5.21 (m, 1 H), 4.90 (ddd, J = 12.0, 9.8, 4.7 Hz, 1 H), 4.41-4.38 (m, 2 H), 4.16-4.04 (m, 4 H), 3.92 (dd, J = 9.6, 5.5 Hz, 1 H), 3.83-3.79 (m, 4 H), 3.73 (dt, J = 12.9, 6.4 Hz, 1 H), 3.40 (dt, J = 13.2, 6.6 Hz, 1 H), 3.11 (s, 1 H), 2.59 (dd, J = 12.8, 4.6 Hz, 1 H), 2.13 (s, 3 H), 2.11 (s, 3 H), 2.03 (s, 3 H), 2.02-2.00 (m, 1 H), 1.94 (s, 3 H), 1.88 (s, 3 H), 1.63-1.56 (m, 2 H), 0.89 (t, J = 7.4 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.97, 170.96, 170.27, 170.23, 168.07, 166.06, 166.01, 133.16, 133.11, 129.84, 129.79, 129.74, 129.62, 128.36, 128.34, 98.74, 96.52, 72.90, 71.26, 70.11, 68.03, 53.07, 49.41, 23.20, 22.65, 21.07, 20.86, 20.80, 10.57; HRMS (LTQ-Orbitrap XL, positive) Anal. Calculated for $C_{43}H_{53}NO_{20}Na_1$ $[M+Na]^+$: 926.3053, found 926.3019.



Propyl 3-*O*-benzoyl-6-*O*-benzyl-α-D-galactopyranoside (4)

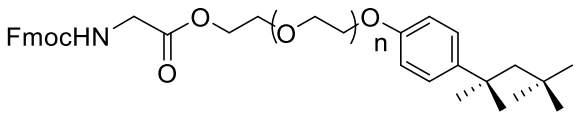
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09-8.07 (m, 2 H), 7.57 (t, J = 7.5 Hz, 1 H), 7.44 (t, J = 7.8 Hz, 2 H), 7.38-7.29 (m, 5 H), 5.27 (dd, J = 9.7, 3.8 Hz, 1 H), 5.16 (d, J = 3.8 Hz, 1 H), 4.66-4.58 (m, 2 H), 4.17-4.15 (m, 2 H), 4.05 (t, J = 4.9 Hz, 1 H), 3.85-3.77 (m, 2 H), 3.69-3.64 (m, 1 H), 3.41-3.37 (m, 1 H), 3.16 (s, 1 H), 2.69 (s, 1 H), 1.62-1.55 (m, 2 H), 0.89 (t, J = 7.4 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.97, 137.66, 133.21, 129.88, 129.77, 128.51, 128.35, 127.87, 127.74, 96.59, 73.80, 72.35, 70.65, 70.15, 69.97, 68.70, 68.33, 22.67, 10.55; HRMS (LTQ-Orbitrap XL, positive) Anal. Calculated for $C_{23}H_{28}O_7Na_1$ $[M+Na]^+$: 439.1727, found 439.1735.



4-Methoxyphenyl 2,4,6-tri-*O*-benzyl-3-*O*-(Methyl 5-acetamide-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-β-D-galactopyranoside (7)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 (d, J = 7.2 Hz, 2 H), 7.35-7.25 (m, 13 H), 7.03-6.99 (m, 2 H), 6.80-6.76 (m, 2 H), 5.49-5.46 (m, 1 H), 5.29 (dd, J = 8.2, 2.0 Hz, 1 H), 5.17-5.15 (m, 1 H), 4.96-4.83 (m, 5 H), 4.54-4.42 (m, 3 H), 4.36 (dd, J = 12.5, 2.6 Hz, 1 H), 4.24 (dd, J = 9.9, 2.9 Hz, 1 H), 4.05 (q, J = 10.4 Hz, 1 H), 3.97-3.88 (m, 3 H), 3.76 (s, 3 H), 3.73 (s, 4 H), 3.71-3.69 (m, 2 H), 3.68-3.64 (m, 1 H), 2.53 (dd, J = 13.2, 4.9 Hz, 1 H), 2.11 (s , 3 H), 2.10-2.06 (m, 1 H), 1.99 (s , 3 H), 1.96 (s , 3 H), 1.92 (s , 3 H), 1.88 (s , 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.87, 170.49, 170.25, 169.79, 168.26, 155.03, 151.71, 139.07, 138.96, 138.11, 128.30, 128.05, 128.03, 127.83, 127.68, 127.64, 127.62, 127.23, 127.20, 118.39, 114.37, 102.85, 98.63, 77.43, 76.21, 76.16, 74.86, 74.83, 73.44, 73.37, 72.44, 69.18, 68.89, 68.70, 67.35, 55.61, 52.82, 49.31, 23.18, 21.12, 20.80, 20.70, 20.57*;* HRMS (LTQ-Orbitrap XL, positive) Anal. Calculated for $C_{54}H_{63}NO_{19}Na_1$ $[M+Na]^+$: 1052.3886, found 1052.3901.

Experiments in Chapter III

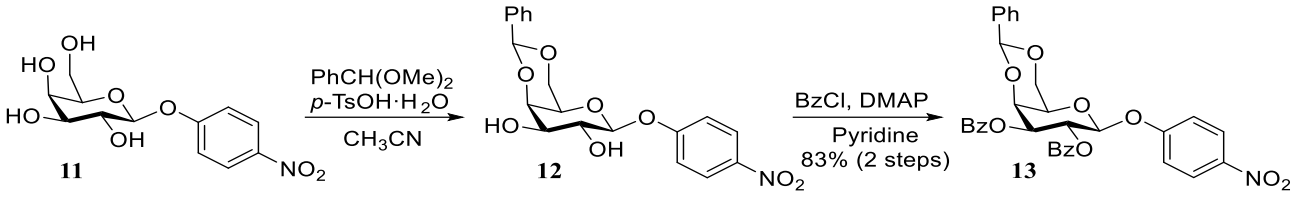


Fmoc-glycine linked with Triton X-100 (49)

To a solution of Triton X-100 (50 mg, 77 μmol), Fmoc-glycine (28 mg, 93 μmol), DMAP (2 mg, 19 μmol) in dry CH_2Cl_2 (1 mL) was added DIPC (30 μL, 0.193 mmol) at 0°C. The reaction was stirred at rt for 22 h.

Affinity Separation:

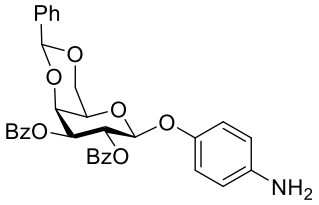
The ArgoPore-NH_2 beads (1.5 g) were placed in a Varian, Bond Elut empty cartridge with frit (6 mL). The column was washed with CH_2Cl_2/MeOH (1:1) and CH_2Cl_2, followed by addition of 10% TFA in CH_2Cl_2 (12 mL) to change the -NH_2 groups on the resin to –NH_3^+. Excess TFA was washed with CH_2Cl_2 followed by addition of the crude reaction mixture dissolved in CH_2Cl_2. The column was washed several times with CH_2Cl_2 to remove excess reagents, then the product was desorbed using CH_2Cl_2/MeOH (1:1) to give compound **49** (62 mg, 87%).



4,6-*O*-Benzylidene-2,3-di-*O*-benzoyl-4-nitrophenyl-β-D-galactopyranoside (13)

To a solution of commercially available 4-nitrophenyl-β-D-galactopyranoside **11** (1 g, 3.31 mmol) in dry CH_3CN (33.1 mL) was added benzaldehyde dimethyl acetal (1.52 g, 9.96 mmol) and *p*-TsOH·H_2O (0.158 g, 0.83 mmol). The mixture was stirred at rt for 24 h, then quenched with Et_3N followed by concentration in vacuo. The dry crude mixture was dissolved in CH_2Cl_2 and washed thrice with saturated $NaHCO_3$ (aq), then thrice with saturated NaCl (aq). The organic layer was dried over anhydrous Na_2SO_4 followed by concentration in vacuo with toluene azeotrope to get crude compound **12.**

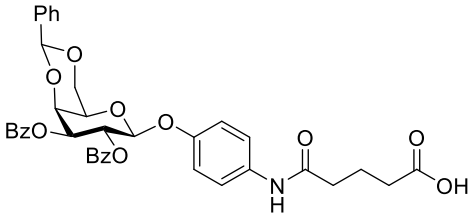
To a solution of **12** in dry pyridine (25 mL) was added benzoyl chloride (1.54 mL, 13.25 mmol) and stirred followed by the addition of DMAP (40 mg, 0.331 mmol) at 0°C. The reaction mixture was stirred at rt for 11.5 h followed by extraction using EtOAc and 1M HCl (aq). The organic layer was washed thrice with saturated $NaHCO_3$ (aq), then twice with saturated NaCl (aq), and dried over anhydrous Na_2SO_4. After drying in vacuo with toluene azeotrope, the crude mixture was recrystallized in EtOH to give **13** (1.65 g, 83% over 2 steps) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.16-8.13 (m, 2 H), 8.01-7.99 (m, 2 H), 7.96-7.94 (m, 2 H), 7.54-7.50 (m, 4 H), 7.40-7.35 (m, 7 H), 7.13-7.10 (m, 2 H), 6.17 (dd, J = 10.4, 8.0 Hz, 1 H), 5.60 (s, 1 H), 5.50-5.46 (m, 2 H), 4.70 (d, J = 3.5 Hz, 1 H), 4.45 (dd, J = 12.6, 1.3 Hz, 1 H), 4.21 (dd, J = 12.6, 1.6 Hz, 1 H), 3.91 (s, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.1, 161.5, 143.1, 133.6, 130.0, 129.7, 129.1, 128.9, 128.5, 128.4, 128.2, 126.2, 125.7, 117.0, 101.0, 99.1, 73.2, 68.5, 67.1; HRMS (LTQ-Orbitrap XL, positive) Anal. Calculated for $C_{33}H_{27}NO_{10}Na_1$ $[M+Na]^+$: 620.1527, found 620.1536.



4,6-*O*-Benzylidene-2,3-di-*O*-benzoyl-4-aminophenyl-β-D-galactopyranoside (14)

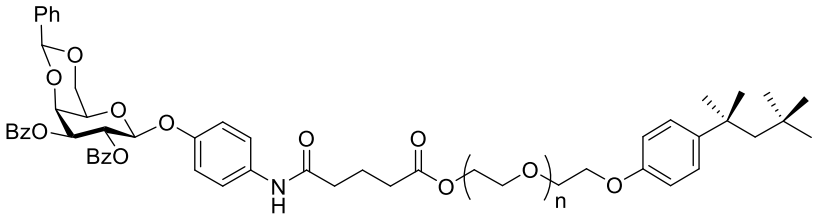
Zinc powder (3 g) in deionized H_2O (20 mL) was sonicated for 40 min. followed by the addition of $CuSO_4$ (aq). The precipitate was filtered and added immediately to a solution of **13** (100 mg, 0.167 mmol) in AcOH/THF (1:1, 10 mL). The reaction mixture was stirred at rt for 1 h, followed by filtration then extraction

using EtOAc and saturated $NaHCO_3$ (aq). The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo to give **14** (93.7 mg, 99%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01-7.98 (m, 4 H), 7.55-7.48 (m, 4 H), 7.40-7.35 (m, 7 H), 6.89-6.86 (m, 2 H), 6.57-6.53 (m, 2 H), 6.09-6.05 (m, 1 H), 5.57 (s, 1 H), 5.39 (dd, J = 10.5, 3.6 Hz, 1 H), 5.14 (d, J = 8.2 Hz, 1 H), 4.64-4.63 (m, 1 H), 4.44 (dd, J = 12.3, 1.6 Hz, 1 H), 4.15 (dd, J = 12.3, 1.7 Hz, 1 H), 3.75-3.74 (m, 1 H), 3.48 (s, 2 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.2, 142.4, 137.5, 130.0, 129.7, 129.0, 128.4, 128.3, 128.1, 126.3, 119.8, 115.8, 101.0, 73.5, 69.1, 68.9; HRMS (LTQ-Orbitrap XL, positive) Anal. Calculated for $C_{33}H_{29}NO_8Na_1$ $[M+Na]^+$: 590.1785, found 590.1796.



5-oxo-5-((4-((4,6-*O*-Benzylidene-2,3-di-*O*-benzoyl-β-D-galactopyranosyl)oxy)phenyl)amino)pentanoic acid (15)

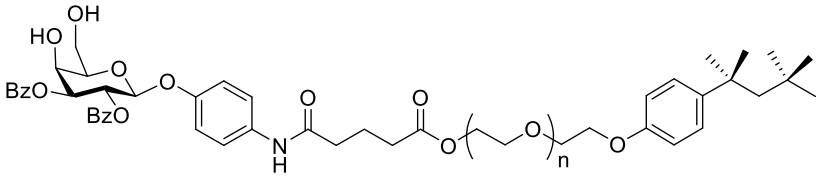
To a solution of **14** (73 mg, 0.128 mmol) in dry CH_2Cl_2 (1 mL) was added glutaric anhydride (22 mg, 0.193 mmol). The mixture was stirred at rt for 1h then the precipitate was filtered, transferred to a vial and further dried in vacuo to give **15** (42 mg, 48%) as a white powder. ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 9.82 (s, 1 H), 7.89-7.87 (m, 2 H), 7.84-7.82 (m, 2 H), 7.61-7.58 (m, 2 H), 7.49-7.36 (m, 11 H), 6.96-6.94 (m, 2 H), 5.75-5.67 (m, 4 H), 4.72 (d, J = 2.6 Hz, 1 H), 4.21 (s, 2 H), 4.15 (s, 1 H), 2.30 (t, J = 7.5 Hz, 2 H), 2.24 (t, J = 7.3 Hz, 2 H), 1.80-1.75 (m, 2 H); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 191.3, 174.1, 170.4, 165.0, 164.9, 152.2, 138.1, 134.4, 133.8, 133.7, 131.8, 129.1, 128.8, 128.7, 128.3, 128.1, 126.0, 120.4, 116.8, 114.5, 99.5, 98.2, 73.1, 72.0, 69.2, 68.0, 66.0, 55.7, 35.3, 33.0, 20.5; HRMS (LTQ-Orbitrap XL, positive) Anal. Calculated for $C_{38}H_{35}NO_{11}Na_1$ $[M+Na]^+$: 704.2102, found 704.2115.



Triton-tagged monosaccharide (16)

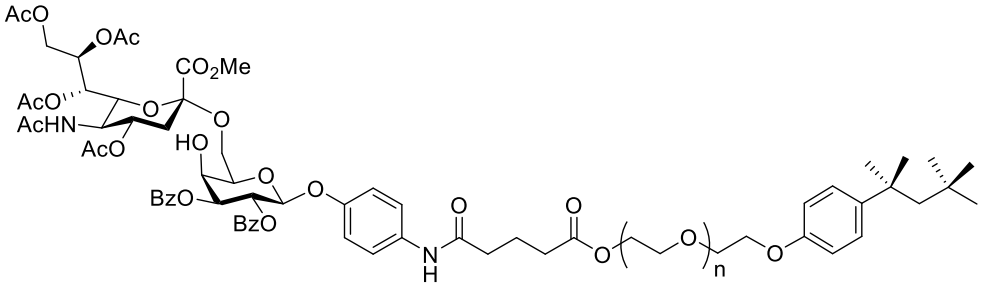
To a solution of Triton X-100 (15.8 mg, 24 μmol) in dry DMF (1 mL) was added **15** (20 mg, 29 μmol), HOBt (4.9 mg, 36 μmol) and WSCD·HCl (6.9 mg, 36 μmol). Then Et_3N (10 μL, 72 μmol) was added at 0°C. The reaction mixture was stirred at rt for 22 h followed by extraction using $CHCl_3$ and saturated $NaHCO_3$

(aq). The organic layer was washed twice with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified using silica-gel column chromatography (500 mg, $CHCl_3$/MeOH = 10:1) to give **16** (21.2 mg, 67%) as a clear oil.



Benzylidene-deprotected triton-tagged monosaccharide (17)

To **16** (21.2 mg, 16 μmol) was added a mixture of TFA-H_2O-CH_2Cl_2 (3:2:10) and stirred at rt for 4 h. The reaction mixture was diluted with $CHCl_3$ and washed with vitamin C buffer solution. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo followed by purification using ArgoPore-NH_2. The ArgoPore-NH_2 beads (1.5 g) were placed in a Varian, Bond Elut empty cartridge with frit (6 mL). The column was washed with CH_2Cl_2/MeOH (1:1) and CH_2Cl_2, followed by addition of 10% TFA in CH_2Cl_2 (12 mL) to change the -NH_2 groups on the resin to –NH_3^+. Excess TFA was washed with CH_2Cl_2 followed by addition of the crude reaction mixture dissolved in CH_2Cl_2. The column was washed several times with CH_2Cl_2 to remove excess reagents, then the product was desorbed using CH_2Cl_2/MeOH (1:1). The product was diluted with $CHCl_3$ and washed with citric acid to remove excess TFA. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo to give **17** (13.3 mg, 67%) as an oil.

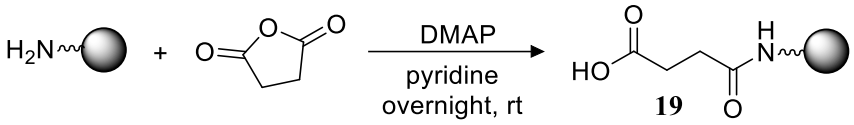


Triton-tagged sialic acid-containing disaccharide (18)

To a solution of **17** (12.7 mg, 10.4 μmol) in dry EtCN (300 μL), **1** (9.09 mg, 15.6 μmol), and activated molecular sieves 5Å was added. ICl (4.89 mg, 30.1 μmol) and $In(OTf)_3$ (11.68 mg, 20.8 μmol) were added at -85°C and the reaction mixture was stirred for 3 h. This was diluted with $CHCl_3$ (2 mL) and warmed to rt followed by the addition of vitamin C buffer. After extraction by $CHCl_3$, the organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The crude mixture was diluted with CH_2Cl_2 and passed through an ArgoPore-NH_2 column previously treated with TFA. After washing the excess reagents with CH_2Cl_2, the triton-tagged disaccharide was desorbed using CH_2Cl_2/MeOH (1:1). The product was diluted with $CHCl_3$ and

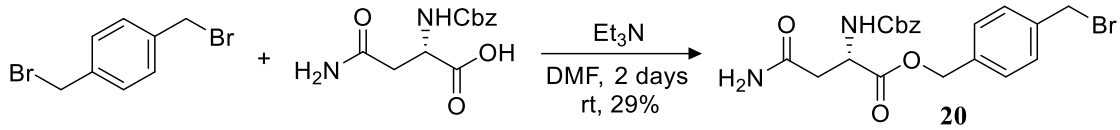
washed with citric acid to remove excess TFA. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo to give **18** (51% based on ^1H NMR) as an oil.

Experiments in Chapter IV



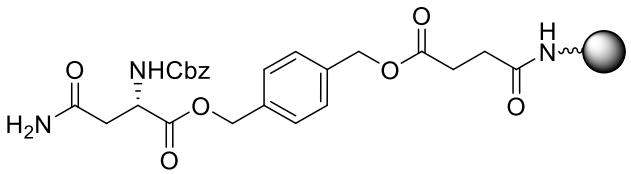
Succinic anhydride functionalized JandaJel beads (19)

JandaJel beads (100 mg, 0.1mmol) were placed in Varian, Bond Elut empty cartridge with frit (6 mL), washed with CH_2Cl_2 (5 times) to expand, then washed further with dry pyridine thrice. A solution of succinic anhydride (50 mg, 0.5 mmol), and DMAP (3 mg, 25 µmol) in dry pyridine (2 mL) was added to the JandaJel beads and shaken at rt for 22 h. The solution was filtered off and the resin was washed with CH_2Cl_2, and MeOH, alternatively (each 2 mL, 2 min., 15 times) to give **19**.



Asparagine linked with bromomethyl benzene (20)

To a solution of 1,4-bis(bromomethyl)benzene (132 mg, 0.5 mmol) in dry DMF (2 mL) was added Et_3N (69.7 µL, 0.5 mmol), and L-asparagine (133.13 mg, 0.5 mmol). The reaction was stirred at rt for 2 days. The reaction mixture was diluted with $CHCl_3$ and washed with H_2O. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. Purification was done by silica-gel column chromatography (5 g, toluene/EtOAc = 45:1) to give **20** (65.6 mg, 29%).



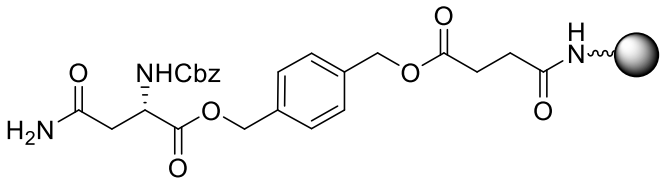
Benzyl ester linked asparagine on JandaJel beads (21)

***Synthesis*:**

To compound **19** (25 mg, 25 µmol) was added **20** (41.7 mg, 92.5 µmol) in DMF (2 mL) followed by Et_3N (16.7 µL, 30 µmol). The reaction mixture was shaken at rt for 20 h, then the solution was filtered off. The resin was washed with CH_2Cl_2, and MeOH, alternatively (each 2 mL, 2 min., 15 times) to give **21**.

Cleavage:

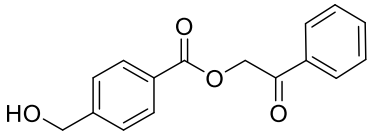
Compound **21** was washed twice with THF, then THF (1 mL) was added followed by 2N NaOH (500 μL). The reaction mixture was shaken at rt for 20 h. The solution was filtered off and collected while the resin was washed some more with THF (1 mL, 8 times). The filtered solution and THF washings were combined, neutralized using 1M HCl (aq), and concentrated in vacuo. Silica-gel column chromatography (3 g, hexane:EtOAc = 1:2) was done to give *p*-xylylene glycol (1.2 mg, 35%).



(25)

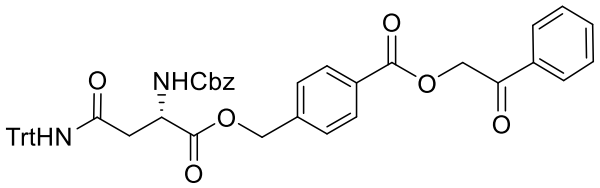
JandaJel beads (100 mg, 0.1 mmol) were washed with CH_2Cl_2, then DMF (each 2 mL, 3 min., 3 times). A solution of **22** (71.5 mg, 0.3 mmol), and HOBt (40.5 mg, 0.3 mmol) in dry DMF (2 mL) was added DIPC (47 μL, 0.3 mmol) at 0°C and stirred. After 30 min., this was added to the previously washed JandaJel resin, and the mixture was shaken at rt for 17 h. The solution was filtered off, and the resin was washed with DMF, MeOH, and CH_2Cl_2, alternatively (each 2 mL, 2 min., 5 times) to give **23**. Bromophenol blue test: JandaJel beads = violet, **23** = pale yellow.

N-Carbobenzyloxy-β-trityl-L-asparagine (153 mg, 0.3 mmol), and MSNT (89 mg, 0.3 mmol) in dry CH_2Cl_2 (2 mL) was added 1-methylimidazole (24 μL, 0.3 mmol) at rt. This was added to **23**, and the mixture was shaken at rt for 22 h. The solution was filtered off and the resin was washed with CH_2Cl_2, then DMF (each 2 mL, 3 min., 5 times). 2% TFA in CH_2Cl_2 (2 mL) was added, the mixture was shaken at rt for 10 min, and then, the solution was filtered off. This step was done twice. The resin was washed with CH_2Cl_2 (2 mL, 3 min., 5 times) to give **25**.



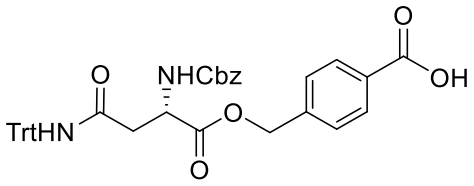
2-oxo-2-phenylethyl 4-(hydroxymethyl)benzoate (26)

To a solution of 2-bromoacetophenone (0.98 g, 4.95 mmol) in dry DMF (13 mL) was added Et_3N (0.46 mL, 3.29 mmol) and 4-(hydroxymethyl)benzoic acid (0.5 g, 3.29 mmol). The reaction mixture was stirred at rt for 3 h, then concentrated in vacuo. Silica-gel column chromatography (15 g, $CHCl_3$:MeOH = 25:1) was done to get **26** (0.81 g, 91%).



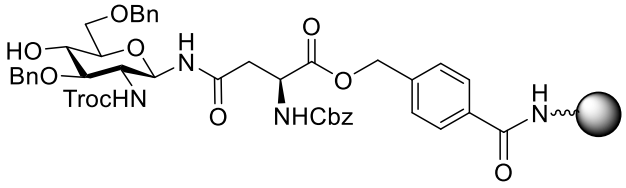
2-oxo-2-phenylethyl 4-(((*N*^{2}-((benzyloxy)carbonyl)-*N*^{4}-trityl-L-asparaginyl)oxy)methyl)benzoate (27)

To a solution of *N*-carbobenzyloxy-β-trityl-L-asparagine (1.12 g, 2.21 mmol), HOBt (0.3 g, 2.21 mmol), **26** (0.2 g, 0.74 mmol), and DMAP (23 mg, 0.18 mmol) in dry DMF (3 mL) was added WSCD·HCl (0.42 g, 2.21 mmol) at 0°C. The reaction mixture was stirred at rt for 20 h, then diluted with $CHCl_3$, and washed with water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. Silica-gel column chromatography (6 g, toluene:EtOAc = 1:1) was done to get **27** (0.4 g, 73%).



Asparagine with benzyl ester linker (28)

Zinc powder (4 g) in deionized H_2O (20 mL) was sonicated for 1 h followed by the addition of $CuSO_4$ (aq). The precipitate was filtered and added immediately to a solution of **27** (1.13 g, 1.48 mmol) in AcOH/THF (1:1, 9.4 mL). The reaction mixture was stirred at rt for 3 h, followed by filtration, and the filtrate was concentrated in vacuo. Silica-gel column chromatography (60 g, toluene:EtOAc:MeOH = 1:1:0.25) was done to get **28** (0.88 g, 92%).



Monosaccharide linked with asparagine on JandaJel beads (32)

Synthesis:

JandaJel beads (100 mg, 0.1 mmol) was washed with CH_2Cl_2, and DMF (each 2 mL, 3 min., 3 times). To a solution of **28** (192.8 mg, 0.3 mmol), and HOBt (40.5 mg, 0.3 mmol) in dry DMF (2 mL) was added DIPC (47 µL, 0.3 mmol) at 0°C. This was then added to the JandaJel resin, and the mixture was shaken at rt for 18.5 h. The solution was filtered off, and then resin was washed with DMF, MeOH, and CH_2Cl_2, alternatively (each 2 mL, 3 min., 5 times) to give **29**.

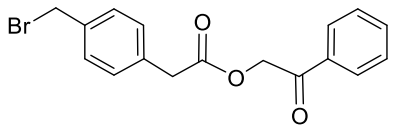
2% TFA in CH_2Cl_2 was added to **29**, the mixture was shaken at rt for 10 min., then, the solution was filtered off. This step was done twice. The resin was washed with CH_2Cl_2 (2 mL, 3 min., 5 times) then dried in vacuo to give **30**.

N-(phenyl)trifluoroacetimidate donor (0.196 g) in dry CH_2Cl_2 (2 mL), and activated moleculare sieves 4Å (20 beads) were added to **30**. TMSOTf (18.10 µL) was added to the reaction mixture at -78°C, and the mixture was shaken. After 20 min., the reaction mixture was warmed to rt and shaken for 17 h. The solution was filtered off, the molecular sieves removed, and the resin was washed with CH_2Cl_2 and MeOH, alternatively (2 mL, 3 min., 10 times) to give **31**.

15% Et_3N in CH_2Cl_2 (2 mL) was added to **31**, and the mixture was shaken at rt for 4 h. The solution was filtered off and collected. The resin was washed with CH_2Cl_2 (2 mL, 3 min., 5 times). The filtered solution, and CH_2Cl_2 washings were combined then used for UV analysis of the 9-methylene-9*H*-fluorene chromophore. On the other hand, the resin was washed further with DMF, CH_2Cl_2 and MeOH, alternatively (2 mL, 3 min., 5 times) to give **32**.

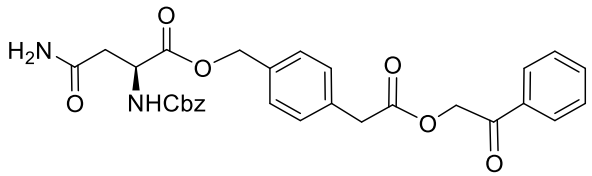
Cleavage:

4M NaOH (27 µL) in 1,4-dioxane (1.92 mL) was added to **32** and the reaction mixture was shaken at rt for 1 h. The solution was filtered off and collected. The resin was washed with 1,4-dioxane (1 mL, 2 min. 3 times). The filtered solution and washings were combined and concentrated in vacuo to give **33**.



2-oxo-2-phenylethyl 2-(4-(bromomethyl)phenyl)acetate (34)

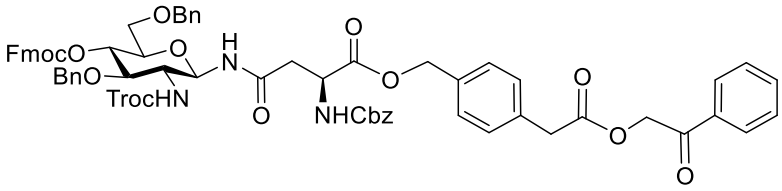
To a solution of 2-bromoacetophenone (1.40 g, 7.03 mmol) in dry DMF (34.9 mL) was added KF (1.09 g, 18.8 mmol), and 4-(bromomethyl)phenylacetic acid (2 g, 8.73 mmol).The reaction mixture was stirred at rt for 25 h, then filtered, and concentrated in vacuo. Silica-gel column chromatography (60 g, toluene:EtOAc = 45:1) was done to get **34** (1.5 g, 62%).



4-(2-oxo-2-(2-oxo-2-phenylethoxy)ethyl)benzyl ((benzyloxy)carbonyl)-L-asparagine (35)

To a solution of *N*-carbobenzyloxy-L-asparagine (1 g, 3.76 mmol) in MeOH (23 mL) and water (25 mL) was added 20% $CsCO_3$ while stirring at 0°C until pH = 7. Then, the solution was dried in vacuo. The residue

was redissolved in dry DMF (10 mL) and **34** (1.30 g, 3.74 mmol) was added. The reaction mixture was stirred at rt for 1 h, then concentrated in vacuo. Silica-gel column chromatography (30 g, hexane:EtOAc = 1:3) was done to get **35** (0.92 g, 46%).



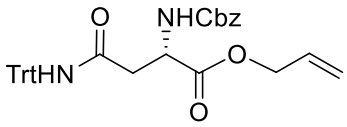
Monosaccharide linked with asparagine (36)

Synthesis:

N-(phenyl)trifluoroacetimidate donor (219 mg, 0.236 mmol) and **35** (200 mg, 0.376 mmol) were lyophilized for 3h followed by the addition of dry CH_2Cl_2 (4.64 mL), and activated moleculare sieves 4Å. TMSOTf (5.67 µL, 31.4 µmol) was added to the reaction mixture at -78°C, and stirred. After 20 min., the reaction mixture was warmed to rt and stirred for 43 h. The reaction was quenched by saturated $NaHCO_3$ (aq), then filtered. Extraction was done using EtOAc, and the organic layer was washed with saturated NaCl (aq), then dried over anhydrous Na_2SO_4, and concentrated in vacuo. Silica-gel column chromatography (6 g, toluene:EtOAc = 1.5:1) was done to get **36** (52.5 mg, 18%).

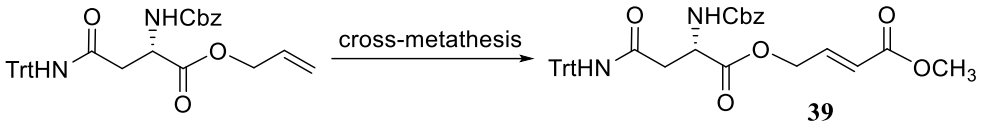
Cleavage:

To a solution of **36** (45 mg, 35 µmol) in THF/water (1:0.5, 1 mL) was added $LiOH{\cdot}H_2O$ (4.45 mg, 0.106 mmol), and the mixture was stirred at rt for 2 d. The reaction was quenched using Dowex H^+ 50WX8. Silica-gel column chromatography (1 g, $CHCl_3$:MeOH = 2:1) was done to get **37** (6 mg, 29%).



***N*-carbobenzyloxy-β-trityl-L-asparagine allyl ester (38)**

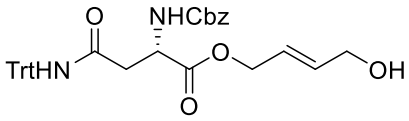
To a solution of *N*-carbobenzyloxy-β-trityl-L-asparagine (1 g, 1.97 mmol) in dry DMF (2 mL) was added allyl bromide (0.18 mL, 2.06 mmol) at rt followed by Et_3N (0.287 mL, 2.06 mmol) at 0°C. The reaction mixture was stirred at rt for 3 h, then quenched by 1M HCl (aq). Extraction was done using EtOAc and the organic layer was washed with saturated $NaHCO_3$ (aq), and brine, dried over anhydrous Na_2SO_4, then concentrated in vacuo. Silica-gel column chromatography (45 g, $CHCl_3$:MeOH = 10:1) was done to get **38** (0.98 g, 90%).



39

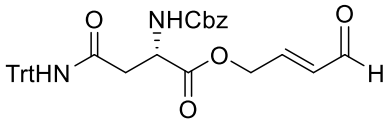
Representative procedure for cross-metathesis reactions.

A mixture of **38** (100 mg, 0.182 mmol), second generation Grubbs catalyst (12 mg, 15 μmol), and methyl acrylate (3.64 mL) was refluxed for 7 h, then cooled to rt, and concentrated in vacuo. Silica-gel column chromatography (6 g, toluene:EtOAc = 2:1) was done to get **39** (30 mg, 27%).



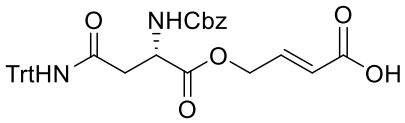
(E)-4-hydroxy-2-butenyl N^2-((benzyloxy)carbonyl)-N^4-trityl-L-asparagine (40)

To a solution of *N*-carbobenzyloxy-β-trityl-L-asparagine (1.7 g, 3.34 mmol), HOBt (1.35 g, 10.03 mmol), DMAP (49 mg, 0.4 mmol) in dry DMF (13.36 mL) was added 2-butene-1,4-diol (0.82 mL, 10.03 mmol), and WSCD·HCl (1.92 g, 10.03 mmol). The reaction mixture was stirred at rt for 4 h, then diluted with $CHCl_3$, and washed with H_2O. The organic layer was dried over anhydrous Na_2SO_4, then concentrated in vacuo. Silica-gel column chromatography (60 g, toluene:EtOAc = 1:1) was done to get **40** (1.41 g, 73%).



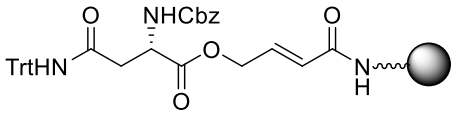
(E)-4-oxo-2-butenyl N^2-((benzyloxy)carbonyl)-N^4-trityl-L-asparagine (41)

To a solution of **40** (100 mg, 0.17 mmol) in CH_2Cl_2 (1.0 mL) was added DMP (81 mg, 0.19 mmol), and the mixture was stirred at rt for 30 min. The reaction mixture was diluted with EtOAc followed by the addition of $Na_2S_2O_3$ in saturated $NaHCO_3$ aqueous solution, stirring rapidly for 10 min. until the mixture became clear. The aqueous layer was extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ (aq), H_2O, and saturated NaCl (aq). The organic layer was dried over anhydrous Na_2SO_4, then concentrated in vacuo. Silica-gel column chromatography (3 g, toluene:EtOAc = 2:1) was done to get **41** (87.6 mg, 88%).



(E)-4-((*N*^{2}-((benzyloxy)carbonyl)-*N*^{4}-trityl-L-asparaginyl)oxy)-2-butenoic acid (42)

To a solution of **41** (1.2 g, 2.08 mmol) in *t*-BuOH (73.43 mL) and H_2O (18.36 mL) was added NaH_2PO_4 (0.62 g, 5.2 mmol), 2-methyl-2-butene (2.76 mL, 26 mmol), and $NaClO_2$ (1.41 g, 15.6 mmol). The mixture was stirred at rt for 28 h, then quenched using 1M HCl until pH = 4. Extraction was done using EtOAc, then the organic layer was washed with saturated NaCl (aq), dried over anhydrous Na_2SO_4, and concentrated in vacuo. Silica-gel column chromatography (40 g, toluene:EtOAc = 2:1) was done to get **42** (0.78 g, 63%).



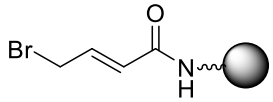
Allyl-linked asparagine on JandaJel beads (43)

Synthesis:

JandaJel beads (100 mg, 0.1 mmol) were washed with CH_2Cl_2 and DMF (2 mL, 2 min., 2 times). To this a solution of **42** (195 mg, 0.33 mmol), and HOBt (60 mg, 0.45 mmol) in dry DMF (1 mL) was added at rt followed by the addition of a solution of HBTU (171 mg, 0.45 mmol), and Et_3N (62 µL, 0.45 mmol) in dry DMF (1 mL) at 0°C. The mixture was shaken at rt for 25 h. The solution was filtered off, and the resin was washed with DMF, MeOH, and CH_2Cl_2, alternatively (each 2 mL, 2 min., 3 times) to give **43**.

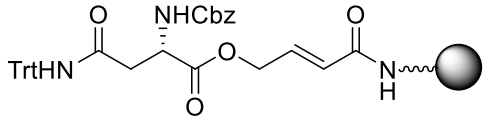
Cleavage:

Resin-bound **43** (50 mg, 50 µmol) was washed with CH_2Cl_2, and THF (2 mL, 2 min., 3 times). To this dry THF (0.75 mL) was added, and the mixture was shaken for 2 min. Then, a solution of $Pd(OAc)_2$ (7 mg, 30 µmol), PPh_3 (37 mg, 0.143 mmol) in dry acetone (0.75 mL) was added, and the mixture shaken. Lastly, sodium-2-ethylhexanoate (62 mg, 0.38 mmol) in dry acetone (0.5 mL) was added to the reaction, and the mixture was shaken at rt for 13 h. The solution was filtered off and collected. The resin was washed with CH_2Cl_2 and THF (each 1 mL, 1 min., 2 times). The washings were collected and combined with the filtered solution, and diluted with EtOA. This was washed with 10% citric acid and H_2O, then dried over anhydrous Na_2SO_4, and concentrated in vacuo. Preparative thin-layer chromatography ($CHCl_3$:MeOH = 7:1) was done to get *N*-carbobenzyloxy-β-trityl-L-asparagine (14 mg, 56%).



Allyl bromide functionalized JandaJel beads (44)

JandaJel beads (100 mg, 0.1 mmol) were washed with CH_2Cl_2 (2 mL, 2 min., 3 times), then added with CH_2Cl_2:DMF (9:1, 1mL). To this, a solution of 4-bromocrotonic acid (33 mg, 0.2 mmol), and HOBt (27 mg, 0.2 mmol) in dry DMF (0.25 mL) was added, then shaken at rt. Afterwards, DIPC (31 µL, 0.2 mmol) was added, and the mixture was shaken at rt for 24 h. The solution was filtered off and the resin was washed with DMF, and CH_2Cl_2, alternatively (each 2 mL, 2 min., 3 times). This coupling was done twice to give **44**.



Allyl-linked asparagine on JandaJel beads (45)

Synthesis:

To **44** (100 mg, 0.1 mmol) was added activated molecular sieves 4Å (10 beads), and dry DMF (1 mL). The mixture was shaken, and was added with a solution of *N*-carbobenzyloxy-β-trityl-L-asparagine (92 mg, 0.18 mmol), KI (30 mg, 0.18 mmol), DIEA (31 µL, 0.18 mmol) in dry DMF (0.5 mL). The mixture was shaken at 40° for 24 h, then the molecular sieves were removed followed by washing with DMF, MeOH, and CH_2Cl_2, alternatively (each 2 mL, 2 min., 3 times). This coupling was done twice to give **45**.

Cleavage:

To **45** (50 mg, 50 µmol) was added a solution of $Pd(PPh_3)_4$ (60 mg, 50 µmol), morpholine (2 µL, 22 µmol), and CuI (1 mg, 5 µmol) in CH_2Cl_2:H_2O (49:1, 1.5 mL). The mixture was shaken at rt for 24 h. The solution was filtered off and collected, while the resin was washed with CH_2Cl_2 (1 mL, 2 min., 3 times). The filtered solution and washings were combined, washed with 10% citric acid then extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, and concentrated in vacuo. Preparative thin-layer chromatography (1 mm, $CHCl_3$:MeOH = 4:1) was done to get *N*-carbobenzyloxy-β-trityl-L-asparagine (13 mg, 50%).

Acknowledgment

The author wishes extend her gratitude to the following people who have contributed so much in the completion of this work:

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